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A QUANTITATIVE STUDY OF THE MICROÖRGANIC POPULATION OF A HEMLOCK AND A DECIDUOUS FOREST SOIL

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The research which has been done in the field of soil bacteriology is comparatively recent, falling within the last half century. Among the quantitative investigations are those in which the organisms have been studied in their relation to depth, moisture, hydrogen-ion, concentration and seasonal effects. The attention has mainly been directed toward agricultural soils because of their economic importance; and it is only recently that forest soils have received much notice. It seems logical to the writer, however, that forest soils, of a virgin nature, might contribute more to our knowledge of soil processes, than those under cultivation; for in the case of virgin soils, microbiological processes have, in a sense, reached an equilibrium. This equilibrium is not broken by plowing, changing of crops, addition of fertilizer, or any of the numerous practices of agriculture which tend to upset or disturb the native microörganic reactions; and thus it would seem to furnish more natural conditions than are possible with cultivated soils.

METHODS

An attempt has been made in the present work to examine and compare certain quantitative and general characteristics of the top and subsoils of a hemlock and a deciduous hardwood forest. The "topsoil" includes the three layers generally known as "litter," "fungous," and "true humus" layers; while the "subsoil" is synonymous with the first layer of "mineral soil." Through the courtesy of the New York Botanical Garden[2] samples were collected once a month over a period of 12 months, from January, 1930, to June, 1930, and from October, 1930, to March, 1931, inclusive, omitting the summer months of July, August, and September.

The hemlock and hardwood forests studied are virgin forests on the grounds of the New York Botanical Garden in New York City. They have been uncut and free from fire for over 100 years (47). The hemlock forest where collec-

[1] This work was done in the Department of Botany, Barnard College, Columbia University, under the direction of Dr. Cornelia L. Carey, to whom the writer is indebted for her suggestion of the problem and her interest and guidance throughout the work.

[2] The writer wishes to thank Dr. Marshall A. Howe and Mr. Percy Wilson of the New York Botanical Garden for their cordial coöperation and assistance in the collection of samples.

tions were made, consists of approximately pure stands of hemlock; the deciduous hardwood forest is the typical climax forest for this region of North America with beech, sugar maple, white oak, witch hazel, and dogwood as the principal vegetation.

The soils studied were sent to the U. S. Department of Agriculture for typing. According to this analysis all of the soils are "a very fine sandy loam," "almost a loam," and closely akin to, if not identical with, the Gloucester loam of this vicinity (70). The results of this analysis are given in table 1.

In general, the methods for soil analysis as set forth by Fred and Waksman (81), and by Waksman (78) were followed in this work. Soil was collected from exactly the same locations each month; all collections were made in sterile cans and sterile trowels were used. Soils were sieved through sterile 3-mm. sieves, and sodium albuminate and synthetic acid media (81) were employed.

TABLE 1

Physical texture

TYPES OF SOILS	COARSE SANDS, 2–0.5 MM.	MEDIUM TO FINE SANDS, 0.5–0.10 MM.	VERY FINE SAND, SILT CLAY, < 0.10 MM.	ACIDITY*	ORGANIC MATTER
	per cent	*per cent*	*per cent*	*pH*	*per cent*
Hemlock topsoil	4	17	79	3.80	50
Hemlock subsoil	6	25	69	4.17	7
Deciduous topsoil	6	23	71	4.79	16
Deciduous subsoil	6	21	73	4.50	8

* The pH given in this analysis is somewhat lower than that obtained by the writer, and is no doubt due to the unavoidable time elapsing between the collection of the samples and their analysis, as the writer has found that a storage of only 24 hours of the hemlock topsoil may change the hydrogen-ion concentration from 5.1 to 4.3. This change is due, in all probability, to the activity of the organisms.

The hemlock topsoil and subsoil were plated out a few hours after collection; likewise, 20-gm. samples of all soils were weighed for moisture percentage determinations and the hydrogen-ion concentration of each soil was taken on the day of collection. The plating of the deciduous topsoil and subsoil could not be carried out, for lack of time, until the following morning (18-hour storage) but since this routine was exactly similar from month to month it was considered that conclusions on the relative numbers of organisms would be valid.

Plates were incubated at room temperature in the dark for one week, counts being made on the third, fifth, and seventh days, and the numbers being expressed on the basis of 1 gm. of soil dried to constant weight at 100°C. The hydrogen-ion concentration was taken on a soil extract made by shaking 5 gm. of soil in 50 cc. of distilled water and filtering through two thicknesses of filter paper. Brom cresol green was used as indicator. The moisture holding capacity was found by saturating a given amount of oven-dry soil

with a known quantity of distilled water, and determining the amount of water absorbed. The temperature data given in the tables were obtained from the U. S. Department of Agriculture Meteorological Data for New York City (71). Both the mean mean monthly temperature and the average of the mean daily temperature for the five days previous to soil collections were used to determine whether any correlation between the temperature and the total numbers of organisms could be distinguished.

DISCUSSION AND RESULTS

Moisture

As may be seen in tables 2 to 5 the lowest moisture content of the soil corresponds to the lowest count of bacteria, except in the hemlock subsoil where the count remained low even after the moisture had increased. The same is true for the fungi in the two deciduous soils, but is not as evident in the hemlock soils. The actinomycetes show a corresponding drop with lack of moisture in both hemlock soils, but some irregularity in the deciduous soils. Drought, therefore, seems to be a limiting factor. It is of interest in this connection to note that the region of collection (as well as the whole country) suffered many weeks of drought previous to the fall (1930) collections. The fungi seem more resistant to low water content than the bacteria and actinomycetes, for although they show a drop in numbers, it is not such a great one as that exhibited by the bacteria and actinomycetes. In general, the reverse statement is true—that at times when the moisture content is higher, the count of the bacteria and actinomycetes is higher also. Here again the numbers of fungi do not show any marked tendency to follow the moisture curve.

These two statements are in accord with the results of Hiltner and Störmer (36), Kruger and Heinze (41), Engberding (18), Conn (11, 12, 13), Russell and Hutchinson (59), Harder (35), Lochhead (43), Snow (62, 63, 64), and Dixon (16, 17); and although Feher and Sommer (27), and Feher (22, 23, 24, 25) do not draw this conclusion, their data as presented would tend to support the foregoing with respect to forest soils.

Fränkel (30), Remy (55), Fischer (28), Brown and Smith (9), Given and Willis (31), Waksman (75), Brown and Halverson (8), Cutler, Crump, and Sandon (15), Erdmann (19), and Newton (49), on the other hand, find no particular correlation between the moisture percentage and the numbers of organisms.

Freezing

The effects of freezing on the numbers of organisms may be seen from tables 2 to 5. On the whole, there was an increase at the beginning of a frozen period (although this is not apparent in the case of the fungi), and the numbers remained higher than the average throughout a frozen period. In general, then, the numbers at this time were high, but not the highest of the year.

TABLE 2

Hemlock topsoil

DATE (1930–1931)	BACTERIA†	FUNGI†	ACTINOMYCES†	pH‡	WATER	MONTHLY RAIN	TEMPERATURE AVERAGE OF 5 DAYS	MEAN MEAN MONTHLY TEMPERATURE
					per cent	*inches*	°F.	°F.
*January	2,630,000	6,900,000	400,000	....	50.0	2.58	47.8	33.6
*February	3,437,500	4,500,000	412,500	4.30	60.0	3.40	34.8	37.0
March	2,529,400	4,302,900	105,880	5.10	57.5	2.16	46.0	39.8
April	6,666,600	4,731,100	215,050	4.70	53.5	2.01	41.8	47.6
May	2,164,920	3,092,740	164,940	4.70	51.5	2.89	59.4	62.6
June	4,905,600	3,547,100	188,675	4.85	47.0	2.92	46.8	73.0
October	868,050	3,506,930	8,845	5.15	28.0	1.76	65.2	62.1
November	1,200,850	2,798,850	55,425	5.10	41.0	5.43	54.4	52.1
December	1,237,185	3,092,740	61,855	5.10	51.5	2.65	35.2	41.3
*January	6,185,530	3,814,415	206,180	5.60	51.5	2.43	32.4	40.5
*February	2,250,000	3,825,000	75,000	5.40	60.0	2.44	29.6	42.1
March	2,620,000	3,273,800	28,450	5.30	58.0	4.74	36.4	40.5

*Soil frozen.

† Counts of organisms represent numbers per gram dry soil.

‡ pH taken after a 6-hour storage except in March, 1930, where a 24-hour storage was unavoidable.

TABLE 3

Hemlock subsoil

DATE (1930–1931)	BACTERIA†	FUNGI†	ACTINOMYCES†	pH‡	WATER	MONTHLY RAIN	TEMPERATURE AVERAGE OF 5 DAYS	MEAN MEAN MONTHLY TEMPERATURE
					per cent	*inches*	°F.	°F.
*January	2,483,600	686,300	196,000	....	23.5	2.58	47.8	33.6
*February	2,030,000	530,825	150,370	4.55	33.5	3.40	34.8	37.0
March	1,830,100	577,460	140,800	5.05	29.0	2.16	46.0	39.8
April	2,922,450	706,120	122,440	4.70	26.5	2.01	41.8	47.6
May	191,250	81,960	0	4.90	26.8	2.89	59.4	62.6
June	606,450	429,675	90,300	4.90	22.5	2.92	46.8	73.0
October	349,715	323,430	14,860	5.20	12.5	1.76	65.2	62.1
November	252,940	235,295	14,705	5.10	15.0	5.43	54.4	52.1
December	278,430	343,790	39,215	5.10	23.5	2.65	35.2	41.3
*January	2,202,610	143,790	91,500	5.50	23.5	2.43	32.4	40.5
*February	623,265	373,960	42,380	5.40	27.8	2.44	29.6	42.1
March	994,595	236,480	148,645	4.90	26.0	4.74	36.4	40.5

* Soil frozen.

† Counts of organisms represent numbers per gram dry soil.

‡ The pH is taken after a 6-hour storage in every case except for March, 1930, where a 24-hour storage was unavoidable.

TABLE 4

Deciduous topsoil

DATE (1930-1931)	BACTERIA†	FUNGI†	ACTINOMYCES†	pH‡	WATER	MONTHLY RAIN	TEMPERATURE AVERAGE OF 5 DAYS	MEAN MEAN MONTHLY TEMPERATURE
					per cent	*inches*	°*F.*	°*F.*
*January	8,360,900	1,278,100	188,000	....	33.5	2.58	47.8	33.6
*February	20,543,200	3,260,900	761,000	4.80	54.0	3.40	34.8	37.0
March	7,086,600	2,047,200	472,400	5.05	36.5	2.16	46.0	39.8
April	9,000,000	1,444,430	555,550	4.80	32.5	2.01	41.8	47.6
May	2,991,100	590,840	295,420	4.80	32.3	2.89	59.4	62.6
June	5,518,200	1,138,660	233,575	5.05	31.5	2.92	46.8	73.0
October	858,800	900,000	76,470	5.20	15.0	1.76	65.2	62.1
November	2,200,000	3,040,000	33,330	5.15	25.0	5.43	54.4	52.1
December	1,566,150	1,786,740	73,530	5.20	32.0	2.65	35.2	41.3
*January	4,223,850	1,044,750	49,250	5.70	33.0	2.43	32.4	40.5
*February	5,811,940	2,478,600	213,675	5.50	41.5	2.44	29.6	42.1
March	7,445,250	1,587,600	620,435	5.30	31.5	4.74	36.4	40.5

* Soil frozen.

† Counts of organisms represent numbers per gram dry soil.

‡ The pH is taken after a 6-hour storage in every case except for March, 1930, where a 24-hour storage was unavoidable.

TABLE 5

Deciduous subsoil

DATE (1930-1931)	BACTERIA†	FUNGI†	ACTINOMYCES†	pH‡	WATER	MONTHLY RAIN	TEMPERATURE AVERAGE OF 5 DAYS	MEAN MEAN MONTHLY TEMPERATURE
					per cent	*inches*	°*F.*	°*F.*
*January	5,986,000	650,350	251,745	....	28.5	2.58	47.8	33.6
*February	5,864,600	548,900	248,120	4.60	33.5	3.40	34.8	37.0
March	5,310,000	655,170	172,400	5.00	27.5	2.16	46.0	39.8
April	2,000,000	866,650	166,665	4.70	25.0	2.01	41.8	47.6
May	3,809,500	721,090	292,515	4.90	26.5	2.89	59.4	62.6
June	6,352,930	1,124,160	209,150	5.05	23.5	2.92	46.8	73.0
October	647,725	250,000	51,140	5.25	12.0	1.76	65.2	62.1
November	807,020	301,755	70,175	5.10	14.5	5.43	54.4	52.1
December	2,373,325	620,000	153,330	5.00	25.0	2.65	35.2	41.3
*January	1,792,245	553,545	116,125	5.50	22.5	2.43	32.4	40.5
*February	1,956,330	253,520	84,510	5.50	29.0	2.44	29.6	42.1
March	913,905	105,960	13,245	5.30	24.5	4.74	36.4	40.5

* Soil frozen.

† Counts of organisms represent numbers per gram dry soil.

‡ The pH is taken after a 6-hour storage in every case except for March, 1930, where a 24-hour storage was unavoidable.

The increase at a time of freezing would tend to confirm the findings of Conn (11, 12, 13), Weber (82), Brown and Smith (9), Given and Willis (31), Harder (35), Waksman (75), Noyes (50), Vass (74), and Brown and Halverson (8), although these investigators differ widely in their theories to account for this increase. Lochhead (43) found only a slight increase in Canadian soil which was frozen consecutively for four or five months, while Vanderleck (72), who also studied Canadian soil under the same conditions, failed to find any noticeable increase in bacterial numbers.

It would seem, then, from the experimental data, that soils which are continuously frozen for months at a time, do not show a marked increase in numbers, or if they show it at the beginning of such a frozen period, the numbers eventually drop to a normal level and remain there. On the other hand, soils which are subject only to north temperate climatic conditions, such as those studied here, and which undergo thawing and freezing rather frequently during the winter months, may exhibit increases in numbers during their frozen periods.

Hydrogen-ion concentration

From data in tables 2 to 5 it will be seen that the small changes in the hydrogen-ion concentration have no apparent relationship to the numbers of organisms present in the soils. Feher (22, 23, 24, 25) and Jensen (38), both working with forest soils of a virgin nature, have found a similar lack of correlation, although Jensen finds a strong positive correlation between the numbers of fungi and of bacteria plus actinomycetes when the hydrogen-ion concentration is 5.5 or below. Bokor (3), working on German forest soils, reports that the total number of bacteria depends chiefly on the hydrogen-ion concentration of the soil, and if this remains constant, the numbers increase in proportion to the organic matter present.

Seasonal effects

The maxima for all three types of organisms seem to come at the same time of year. If the high numbers found at the frozen periods are not considered as true maxima, but as due to some physical cause (such as the breaking up of the clumps, or the rise of organisms with that of the capillary water on freezing), then there is only one maximum. This comes in April at the beginning of the growing season (tables 2 to 5). Two exceptions are to be noted: (*a*) In the deciduous subsoil the rise in bacterial numbers comes later; and (*b*) in the deciduous topsoil the fungi show no apparent April peak. If, as others think, the increase at frozen periods is due to an actual reproductive increase in the numbers of organisms, then two maxima may be seen; that during the frozen period, and the one in April. Interpretation of the increase on a purely mechanical basis seems more logical to the writer, but no definite evidence for either view is available from the present data. It is interesting to note that the June count, in every case except two, leaves the numbers increasing

(tables 2 to 5). It is most unfortunate that the July, August, and September counts could not be made, as investigators who have worked during the summer months on forest soils, Rivas (56), in this country, and Feher (22, 23, 24, 25), in Germany, have found the greatest number of organisms in July or August, and the rise to this peak has begun, in nearly every case, in the month of June. In a number of cases both workers found small peaks in April but these were insignificant in comparison to the July and August maxima.

Temperature

The air temperature has been considered in tables 2 to 5 from two standpoints. In one case, an average of the mean daily temperature for five days previous to collection was taken, in an effort to see whether this might have any bearing on the numbers of organisms at that particular time. In the other case, the mean mean of the monthly temperature was used to determine whether any general effect of the temperature was noticeable. To compare with this, no soil temperatures could be obtained, but in the work of Feher (22, 23, 24, 25) and of Feher and Bokor (26) on forest soils, the soil temperatures and air temperatures are seen to correlate closely, their curves running parallel with only a few degrees difference, so that this would probably be true of any forest soil.

Fabricius and von Felitzen (20), Given and Willis (31), and Brown and Halverson (8) found a definite correlation between the numbers of bacteria and the temperature, in non-forest soils. This was confirmed for forest soils by Feher (22, 23, 24, 25) and Rivas (56).

No such correlation between numbers of organisms and temperature was found in the present work, as may be seen in tables 2 to 5. Other investigators whose findings agree with those given in the tables above are: Fränkel (30), Hiltner and Störmer (36), Engberding (18), and Cutler, Crump, and Sandon (15). Dixon (16, 17) has found (in Australian bush soils) that moisture is more of a controlling factor than temperature as far as the numbers of organisms are concerned, but that a low temperature decreases the numbers.

Depth

Because the topsoil (consisting of the litter, fungus, and true humus layers) and subsoil (or first mineral layer) could be so easily distinguished, the samples were taken according to layers rather than to any definite depth, but the total depth at no time exceeded 35 cm. Tables 2 to 5 reveal that with but few exceptions the numbers of organisms in the two subsoils are less than those in their corresponding topsoils. From this, then, we might conclude that there is a decrease in the numbers of bacteria, fungi, and actinomycetes with an increase in the depth. This is a well-established fact, but the decrease in numbers in the soils here studied may also be due, in part, to the great difference in the chemical nature of the topsoil and its corresponding subsoil. It would seem that since the topsoil has so much more organic matter, and hence more food

and energy sources for the organisms, than its corresponding subsoil, this, rather than a few centimeters difference in depth would cause the drop in the numbers of the different types of microörganisms.

In tables 2 to 5 the moisture content and hydrogen-ion concentration of the various soils are given. The hemlock topsoil, which is the richest in organic matter, has also the highest percentage of moisture in every case; the deciduous topsoil, the next richest in organic matter, has the next highest moisture percentage; and the deciduous subsoil and Hemlock subsoil, having about the same amount of organic matter—much less than the two topsoils—, also have about the same percentage of moisture. (For data on organic matter see table 1.) Thus, the amount of organic matter present seems to have considerable to do with the water holding capacity of the soil.

There is no great difference in hydrogen-ion concentration between any of the four soils, all of them, as soils go, being quite acid, having a range of 4.55–5.7. This acidity is true, in general, for forest soils, and may be partly due to the fungous flora which is abundant in such soils (though, conversely, the fungi flourish because they can stand acidity), and partly to the character of the organic residues in the topsoils. Agricultural soils, for example, have a hydrogen-ion concentration around 7.0, and are richer in bacteria and poorer in fungi than forest soils.

As for the depths of the hemlock and deciduous topsoils, the transition between the two layers (top and subsoil) is much more abrupt in the hemlock soil. Here the topsoil is definitely of a peaty nature, only a few centimeters thick. Of the three layers composing it, the true humus layer is very small—a fraction of a centimeter in most cases—, and the fungous layer predominates. The deciduous topsoil merges with the subsoil more gradually, is deeper, on the whole, than the hemlock topsoil, and is of a "moldy" (leaf mold) rather than a peaty nature. The humus layer is several millimeters thicker than that of the hemlock topsoil, but the fungous layer is not as deep. The layer of litter, though deeper, is less compact than the hemlock litter, so probably does not represent much more material, except after the autumn leaf fall. The two topsoils correspond to the "mull" (mold) and "torf" (peat) soils described for Danish forest soils by Muller (57, p. 70).

Bacteria

Table 6 shows a decided parallelism between the numbers of the bacteria in the hemlock topsoil and hemlock subsoil; and some similarity in the numbers in the deciduous topsoil. The deciduous subsoil, however, fails to show any particular correlation with the other three.

Certain climatic factors, such as air temperature and wind velocity, would have affected these soils similarly. The moisture holding capacity of the soils, however, is not the same. Upon calculation (pp. 326–327) the total moisture holding capacity was found to be as follows: hemlock topsoil 105 per cent, hem-

lock subsoil 73 per cent, deciduous topsoil 85 per cent, deciduous subsoil 73 per cent. Considering the optimum moisture conditions to be 70 per cent of the maximum, as is generally accepted, we have the optimum as: hemlock topsoil 73.3 per cent, hemlock subsoil 51.1 per cent, deciduous topsoil 59.5 per cent, and deciduous subsoil 51.1 per cent. A comparison of these figures with those for percentage of organic matter (table 1) shows that the soils with the most organic matter hold the most moisture. It is evident that the hemlock topsoil with its average moisture for the year of 50.8 per cent comes nearest to filling optimum moisture conditions as far as the organisms are concerned; deciduous topsoil with a year's average of 33.19 per cent comes next; and the two subsoils (deciduous subsoil with an average of 29.2 per cent, and hemlock subsoil with 24.17 per cent) are about equal in this respect.

TABLE 6 ‡

Bacteria†

DATE (1930–1931)	HEMLOCK TOPSOIL	HEMLOCK SUBSOIL	DECIDUOUS TOPSOIL	DECIDUOUS SUBSOIL
*January	2,630,000	2,483,600	8,360,900	5,986,000
*February	3,437,500	2,030,000	20,543,200	5,864,600
March	2,529,400	1,830,100	7,086,600	5,310,000
April	6,666,600	2,922,450	9,000,000	2,000,000
May	2,164,920	191,250	2,991,100	3,809,500
June	4,905,600	606,450	5,518,200	6,352,930
October	868,050	349,715	858,800	647,725
November	1,200,850	252,940	2,200,000	807,020
December	1,237,185	278,430	1,566,150	2,373,325
*January	6,185,530	2,202,610	4,223,850	1,792,245
*February	2,250,000	623,265	5,811,940	1,956,330
March	2,620,000	994,595	7,445,250	913,905

* Soil frozen.

† Counts of organisms represent numbers per gram dry soil.

Now since it is the deciduous subsoil (table 6) which fails to show any parallelism to the other three, and since both the deciduous subsoil and the hemlock subsoil have about the same amount of moisture available to the organisms, we may conclude that moisture, at least, is not responsible for the variations of the deciduous subsoil. Neither is the hydrogen-ion concentration different enough in this soil to be looked upon as a cause.

We must look, then, to some other factor to account for its behavior. This might be: (*a*) a difference in the available food supply for the bacteria in the deciduous subsoil, or (*b*) a difference in the influence of higher plants on the growth of the bacteria in the deciduous subsoil as compared with that in the other soils.

There are in this paper, however, no experimental data to confirm either assumption. They are simply submitted as possible suggestions to account for the dissimilarity of this soil when compared with the others.

The ability of microörganisms to carry on their vital activities depends, as is well known, upon a number of factors, some of which have already been mentioned. There are probably many others of importance about which we have, as yet, little knowledge. Our information is not complete as to the influence of higher plants upon the microörganisms of the soil. The dying of the cells of higher plants affects, of course, the available food supply of the microörganisms by the release of dead organic and inorganic material. Besides this, the plant roots may secrete substances which may hinder or help the organisms. Hansteen-Cranner (34) has shown that plants under ordinary conditions give off from their root cells soluble and insoluble lipoid substances termed "phosphatides." These differ with the plant species and may inhibit or stimulate the microörganisms in that vicinity. Melin (46) has reported a substance which is probably identical to the "phosphatides" of Hansteen-Cranner, from the roots of pine and other coniferous trees. This material has a stimulating action on the growth of certain mycorrhizal fungi. Other investigators have reported on the stimulation of bacterial activity by plant roots; and the excretion of certain salts, organic and inorganic acids, and a number of different sugars has likewise been demonstrated (44, 45, 79). Starkey (65) has made the interesting suggestion that the growth of the higher plants often causes an unequal distribution of the numbers of microörganisms, and that it "may be a major factor in the seasonal fluctuation of the microorganisms in the soil where temperature and moisture do not seem to be related."

As far as the bacteria are concerned, the numbers seem to run parallel to each other in all except the deciduous subsoil. This lack of parallelism cannot be accounted for by any of the factors examined in the present work, but it is suggested that it might be due to a difference in the available food supply of the organisms, or, perhaps, to the influence of the higher plant roots growing at the locations of the soil collections.

It is evident from table 6 that there are, on the average, a greater number of bacteria in the deciduous topsoil than in any other soil. An average for the 12 months of all the bacterial numbers (table 9) shows that the deciduous topsoil has about twice as many bacteria as the deciduous subsoil or the hemlock topsoil (which two soils have about equal numbers) and almost six times as many as the hemlock subsoil. This would suggest that the bacteria play a more active rôle in digestion in the deciduous topsoil than in any of the other soils studied.

Actinomycetes

From table 7, which shows the numbers of actinomycetes, it is evident that the numbers in the two topsoils run somewhat parallel to each other from January to June, 1930, and exactly counter to each other (i.e. if the numbers increase from one month to the next in one soil, they decrease in the other, and vice versa) from October, 1930, to March, 1931. The numbers in the sub-

soils, however, which run counter to each other from January to June, 1930, run nearly parallel from October, 1930, to February, 1931, except that the greatest number in the deciduous subsoil comes one month before that in the hemlock subsoil in the early winter. Whether this is merely accidental, or whether there are complicated factors at work here, is a problem for further study.

There is, however, an interesting point of comparison between the data for the actinomycetes and that presented for the bacteria. An average of the year's counts (table 9) reveals that, almost exactly as in the case of the bacteria, the deciduous topsoil has approximately twice as many actinomycetes as the deciduous subsoil or hemlock topsoil and more than three times as many as the hemlock subsoil, thus giving almost the same comparative re-

TABLE 7

Actinomyces†

DATE (1930–1931)	HEMLOCK TOPSOIL	HEMLOCK SUBSOIL	DECIDUOUS TOPSOIL	DECIDUOUS SUBSOIL
*January	400,000	196,000	188,000	251,745
*February	412,500	150,370	761,000	248,120
March	105,880	140,800	472,400	172,400
April	215,050	122,440	555,550	166,665
May	164,940	0	295,420	292,515
June	188,675	90,300	233,575	209,150
October	8,845	14,860	76,470	51,140
November	55,425	14,705	33,330	70,175
December	61,855	39,215	73,530	153,330
*January	206,180	91,500	49,250	116,125
*February	75,000	42,380	213,675	84,510
March	28,450	148,645	620,435	13,245

* Soil frozen.

† Counts of organisms represent numbers per gram dry soil.

lationship as before. It would seem, then, that as in the case of the bacteria, the actinomycetes were more actively concerned with digestion processes in the deciduous topsoil than in the other soils.

Fungi

From table 8, which shows the numbers of fungi in the four soils, it may be seen that there is some parallelism between the numbers for the hemlock subsoil and those for the deciduous subsoil, except in two points, November, 1930, and February, 1931. It is also readily seen that the numbers of fungi in these two subsoils are approximately equal.

On the other hand, it is evident that the numbers in the hemlock topsoil far exceed those of any of the other four soils, whereas those of the deciduous topsoil lie in an intermediate position. The numbers in the two topsoils show very little similarity to each other, or to those of the two subsoils. Consider-

ing the entirely different chemical nature of the topsoils, due to the different types of vegetation they support, and hence the difference in their organic residues, this is not surprising. The two subsoils, on the other hand, contain about equal amounts of organic matter, and it is possible that the close agreement of the numbers of fungi in the subsoils might be due to this fact, although further work would have to be done to determine this.

The fungous activity is, then, far greater in the hemlock topsoil than in any of the other soils studied; it is next greatest in the deciduous topsoil, and least in the two subsoils. The numerical relationship in the case of the fungi, is not comparable to that of the actinomycetes and bacteria. The hemlock topsoil has about twice as many fungi, on the average, as the deciduous topsoil, seven times as many as the deciduous subsoil, and ten times as many as the hemlock subsoil (table 9).

TABLE 8

Fungi†

DATE (1930–1931)	HEMLOCK TOPSOIL	HEMLOCK SUBSOIL	DECIDUOUS TOPSOIL	DECIDUOUS SUBSOIL
*January	6,900,000	686,300	1,278,100	650,350
*February	4,500,000	530,825	3,260,900	548,900
March	4,302,900	577,460	2,047,200	655,170
April	4,731,100	706,120	1,444,430	866,650
May	3,092,740	81,960	590,840	721,090
June	3,547,100	429,675	1,138,660	1,124,160
October	3,506,930	323,430	900,000	250,000
November	2,798,850	235,295	3,040,000	301,755
December	3,092,740	343,790	1,786,740	620,000
*January	3,814,415	143,790	1,044,750	553,545
*February	3,825,000	373,960	2,478,600	253,520
March	3,273,800	236,480	1,587,600	105,960

* Soil frozen.

† Counts of organisms represent numbers per gram dry soil.

From data in tables 6, 7, and 8 it is apparent that the fungi far outnumber the bacteria and actinomycetes in the hemlock topsoil. The decomposition of organic matter (hemlock needles, twigs etc.) by fungi, is evidently predominant in the hemlock topsoil. This decomposition is thorough and quite rapid, for, as we have said, the topsoil is only a few centimeters deep. The fungi are apparently more successful in the digestion of the particular organic residues found in the hemlock litter than the bacteria are, and hence are more abundant in this soil than the latter. The bacterial numbers on the other hand, predominate in the deciduous topsoil, and probably are responsible for most of the decomposition there. Digestion, no doubt is aided by the fungi, but is largely accomplished by the bacteria with the help of the actinomycetes. The actinomycetes are not numerous in any of the soils, undoubtedly because of their acid character; neither, if we may judge by the numbers, do these or-

ganisms accomplish as much work in the two topsoils as do the fungi and bacteria. Most of the actual decomposition takes place in the "rich" topsoils, but as is shown in tables 6, 7, and 8, the bacteria are the most important agents in the digestion of the material left in the subsoils. They are assisted in this by both the fungi and the actinomycetes.

In order to determine whether there was any constancy or predominance of the types of fungi occurring in the soils from month to month, the synthetic acid medium plates were examined at the end of each count and the types of organisms which predominated were noted.

The species of Penicillium whose conidial stages were different shades of green (macroscopically) could be easily distinguished from species of Trichoderma and Mucor. The typical gray-green colonies of Citromyces were counted from

TABLE 9

Average of numbers for the 12 months

Numbers given per gram dry soil

	HEMLOCK TOPSOIL	HEMLOCK SUBSOIL	DECIDUOUS TOPSOIL	DECIDUOUS SUBSOIL
Bacteria	3,057,960	1,230,450	6,300,500	3,151,130
Fungi	3,948,800	389,100	1,716,490	554,260
Actinomyces	160,190	87,600	297,690	152,430
Citromyces	417,460	25,330	12,090	4,945
Penicillium group excluding Citromyces	553,730	23,400	79,310	35,900

Hemlock topsoil

Average of Penicillium group excluding Citromyces for March, 1930, January, February, March, 1931	631,750
Average of Penicillium group excluding Citromyces for other eight months	376,285
Average of Citromyces group alone for March, 1930, January, February, March, 1931.	576,620
Average of Citromyces group alone for other eight months	233,515

month to month, as well as those of the rest of the Penicillium group mentioned. Since these counts in every case were based purely on macroscopic observations, they cannot be considered more than very roughly accurate. Colonies of each type of fungous were subcultured and examined microscopically from time to time to determine whether macroscopic observations had been valid; and they were found to be so in nearly every case.

Tables 10 and 11 show the comparative numbers for Citromyces and Penicillium in the four soils. These figures run parallel to those for the total numbers of fungi; namely, the hemlock topsoil has the greatest number, the deciduous topsoil the next greatest, and the two subsoils the least, although about equal numbers.

By taking the averages for the 12 counts and comparing them with the averages of the total numbers of fungi (table 9) for the particular soils in

question, it is seen that the Penicillium group, including Citromyces, forms a much higher percentage of the total numbers of organisms in the hemlock topsoil than in any of the other three soils. Possibly this group has some special rôle in the digestive processes in the Hemlock topsoil, although there is in the present data no proof of this.

TABLE 10

Total numbers of Citromyces per gram dry soil

DATE (1930–1931)	HEMLOCK TOPSOIL	HEMLOCK SUBSOIL	DECIDUOUS TOPSOIL	DECIDUOUS SUBSOIL
*January	.....	.....	.....	.....
*February	.....	.....	.....	.....
March	1,294,100	42,260	.....	.....
April	307,530	156,460	26,670	20,000
May	212,370	4,100	22,160	8,850
June	558,490	2,970	8,760	1,700
October	388,890	11,430	0	0
November	277,120	5,880	.....	6,610
December	123,710	785	0	0
*January	309,280	395	4,480	1,940
*February	465,000	2,080	27,350	2,110
March	238,090	27,025	7,300	3,310

* Soil frozen.

TABLE 11

Total numbers of Penicillium group per gram dry soil

DATE (1930–1931)	HEMLOCK TOPSOIL	HEMLOCK SUBSOIL	DECIDUOUS TOPSOIL	DECIDUOUS SUBSOIL
*January	.....	.....	.....	.....
*February	.....	.....	.....	.....
March	541,200	18,310	.....	.....
April	178,500	6,800	71,110	20,000
May	556,700	8,200	73,860	34,020
June	213,200	5,550	48,180	8,630
October	694,400	5,720	35,300	11,370
November	831,400	13,030	.....	175,440
December	536,100	104,580	101,470	13,330
*January	886,600	6,540	128,360	16,780
*February	432,500	18,010	147,010	30,280
March	666,700	47,300	29,200	13,240

* Soil frozen.

From tables 10 and 11 it is apparent that the Penicillium group, including Citromyces, in the hemlock topsoil, form approximately one-fourth of its total fungous population. (See also table 9). In both subsoils their average is roughly one-eighth, whereas in the deciduous topsoil this group forms only one-nineteenth of the total number. Thus it may be seen that in all, except

the hemlock topsoil, the Penicillium group with Citromyces forms a rather insignificant portion of the fungous population.

Actual macroscopic counts of the organisms were not begun until March, 1930, but if the average of the numbers of Citromyces for March, 1930, plus January, February, and March, 1931, be compared with those of the rest of the Penicillium group for the same months, it will be seen that in the hemlock topsoil the Citromyces group is to the Penicillium as 576,600 is to 632,000; or roughly, as 1:1. (table 9). Making the same comparison for the other eight months the relationship is: Citromyces to Penicillium group as 233,500 to 376,300; or roughly, 1:1½ (table 9). Thus not only do the actual numbers of these two groups increase during January, February, and March, but the Citromyces group increases proportionally more than the Penicillium group in the hemlock topsoil. This is interesting and may point to some special function of Citromyces at this time of the year. That this increase in numbers is the result of freezing does not seem the explanation, since the numbers of Trichoderma do not increase during this period, but become less.

Not only do the numbers of Trichoderma become less when the Penicillium group increases, but when the Penicillium group has lost its predominance, in the fall months, Trichoderma again increases greatly, and covers, on the average, 7 of the 9 synthetic acid medium plates poured for any one soil, so that at the end of five days they cannot be counted. This would suggest some mutual seasonal relationship between these two groups of fungi (Penicillium and Trichoderma).

Representatives of the Mucorales were very scarce, and never more than three or four colonies appeared on all the plates from any one soil. They were found, sparsely, in every soil, no soil having particularly more than another. To determine whether the synthetic acid medium was unfavorable to their development, plates were poured (in January, 1931) of Cook's agar (8) as well as the synthetic acid medium plates, since Cook's agar is supposed to favor the growth of Mucorales. More specimens of Mucor came up on the synthetic acid plates than on those of Cook's agar, so it would appear that the medium was not the cause of the lack of Mucors. In defense of this point, Waksman (76) has found that forest and uncultivated soils are poor in Mucorales but rich in specimens of Penicillium and Trichoderma. Jensen (38) in examining 18 forest soils in Denmark reports similar findings.

Besides a scarcity of Mucors, there was but one Aspergillus isolated from the plates during the entire study. Since the conidial stage of the species isolated was not green, it was considered valid to eliminate the species of Aspergillus from the counts of the Penicillium group mentioned in the foregoing. A paucity of Aspergillus in soils from this latitude has been found by other investigators. Waksman (77) and Werkenthin (83) both found that soils in the southern part of the United States were abounding in species of Aspergillus, whereas those of the northern part of the country had predominantly species of Penicillium, Mucor, Trichoderma, and Fusarium, but were poor in Aspergillus.

Waksman (77), Takahashi (67), Fasevoli (21), Paine (51), Dixon (16), Swift (66), and Jensen (38) report that soil fungi common to all parts of the world are species of Penicillium (including Citromyces), Trichoderma, Mucor, and Aspergillus. Some of these writers include species of Fusarium, as well.

Citromyces is typical of the hemlock topsoil. The deciduous top and subsoils seemed to have no "typical fungi" (that is, fungi which occurred in abundance on the plates month after month, but which were not found on plates of the other soils). The hemlock subsoil, however, had two forms which were present in abundance at every collection. The first of these was a gray-green Penicillium which was a bright rose color on the under side (species undetermined). There were always numerous colonies of it on the hemlock subsoil plates, but seldom more than one, if any, on the plates of the other three soils. Its significance, if there is any, offers a problem like that of the Citromyces in the hemlock topsoil, and as suggested for Citromyces, may be concerned with soil digestion processes. The other fungus found so persistently on the hemlock subsoil plates was a sterile white mycelium. Such sterile hyphae have been reported by other workers for forest soils, notably by Traaen (69) for Norwegian soils, by Waksman (76) for American soils, and by Jensen (38) for Danish soils. Waksman thinks that these mycelia are probably the hyphae of mycorrhizal fungi. This might very well be the case here, as the sucking roots of the coniferous trees are known to have an abundant mycorrhizal fungous flora. In any event, their presence in the hemlock subsoil is interesting.

The work of identification of many of these fungous forms is, in itself, a considerable undertaking, as a large proportion of the soil fungi belong to the group "Hyphomycetes," and the numerous sterile hyphae present a baffling problem in that they do not produce conidial stages in the laboratory under ordinary conditions. If the latter are mycorrhiza, as suggested, it is possible that, given other conditions, they might be induced to fruit.

SUMMARY

In general, the greatest numbers of bacteria, fungi, and actinomycetes corresponds to the time of greatest soil moisture. The converse is also true.

Freezing of the soil causes a marked increase in the numbers of organisms, but this increase is not so apparent in the case of the fungi. The frozen counts are high, but not the highest of the year.

The slight variations in the hydrogen-ion concentration have no apparent effect on the total numbers of bacteria, fungi, and actinomycetes.

In general, there is some parallelism between the numbers of bacteria and actinomycetes in each soil. It is not so marked in the case of the fungi.

The seasonal maxima for the three types of organisms come in all soils in April, the beginning of the growing season.

The air temperature seems to have no definite relation to the total numbers of bacteria, fungi, or actinomycetes.

There are, in every case, fewer organisms in the samples of subsoils than in their corresponding topsoils. This may be due to the sandy nature of the subsoils, and to their lack of organic matter, or it may be due purely to the greater depth of these collections.

The deciduous topsoil has twice as many bacteria as the deciduous subsoil or hemlock topsoil (which two soils have about equal numbers) and almost six times as many as the hemlock subsoil.

The deciduous topsoil has twice as many actinomycetes as the deciduous subsoil or hemlock topsoil and nearly three times as many as the hemlock subsoil.

The hemlock topsoil has twice as many fungi as the deciduous topsoil; seven times as many as the deciduous subsoil; and ten times as many as the hemlock subsoil.

These figures show that in the peaty hemlock topsoil the molds are responsible for most of the decomposition; whereas in the deciduous topsoil the bacteria are the main agents in the soil digestion processes, and the fungi are in second place according to numbers. These two topsoils contain, of course, the greatest amounts of undecomposed material.

Most of the work of decomposition is completed in the two topsoils, but in the subsoils that which is left to be done is carried on mainly by the bacteria. The actinomycetes and fungi aid in the process but are not the main agents.

The species of Penicillium including Citromyces show the same numerical relationship to each other in the four soils as do the total numbers of fungi; namely, most numerous in the hemlock topsoil, next most numerous in the deciduous topsoil, and least, but about equally numerous, in the two subsoils. These figures are based purely on macroscopic counts of species of Penicillium or Citromyces whose conidial stages appeared green.

The Penicillium group, including Citromyces, are actually and proportionally more numerous in the hemlock topsoil than in the others. This would suggest that they might play some special rôle in the soil processes of the hemlock topsoil.

The ratio of Citromyces to the Penicillium group (not including Citromyces) in the hemlock topsoil is greater for the four months of March 1930, January, February, and March, 1931, than is the ratio of these organisms during the other eight months that the soils were examined. These averages of numbers check with general notes taken on the plates from month to month, and suggest that Citromyces might have some special function in the hemlock topsoil at this season of the year.

At the time of year when the Citromyces-Penicillium are predominant in the hemlock topsoil, species of Trichoderma fade into the background. The reverse is also true. This would suggest some mutual relationship between Trichoderma and the Penicillium group (including Citromyces).

Species of Penicillium (including Citromyces) and Trichoderma seem to form by far the largest part of the fungous population of the four soils studied. This confirms the work of other forest soil investigators.

Members of the order Mucorales and the genus Aspergillus were found to be very scarce in the soils studied. This also confirms the results of other workers on forest soils.

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THE DIURNAL AND SEASONAL CHANGES IN THE SUGAR CONTENT OF THE SAP AND TISSUE OF POTATO PLANTS AS AFFECTED BY SOIL FERTILIZATION[1]

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One of the most interesting phases of the many chemical studies made of plant tissue is the determination of carbohydrates in the various parts of plants. Most of this work has been directed toward determining how carbon assimilation takes place and how its products are transported from the leaves to other parts of the plant, either for immediate or future use.

The object of this experiment was to study the diurnal and seasonal variations in the sugar content of both leaves and stalks of potato plants for the purpose of determining any differences that might occur as a result of the application of commercial fertilizers to soils growing these potato plants under field conditions, and to determine whether any differences could be correlated with the crop yields. The potato was selected because this plant, through its habits of storing carbohydrates, lends itself very well to this type of investigation.

HISTORICAL

Although Liebig (18) in 1832 advanced the first theory of carbon assimilation in plants, and suggested that organic acids were perhaps the intermediate products, the work of Sachs (28) in 1862, which showed that starch is actually produced within the chlorophyll granule, was really the starting point of investigations concerning carbon assimilation in plants. In 1870, von Baeyer (2) advanced his famous formaldehyde theory, but later Brown and Morris (5), Parkin (24) and Davis, Daish, and Sawyer (8), brought forth evidence to prove that sucrose and not glucose is the first sugar of photosynthesis. However, the work of Dixon and Mason (10), Priestly (25), and more recently that of Clements (6, 7), Baly and Davis (3), and Barton-Wright and Pratt (4), may be interpreted as favoring the von Baeyer theory.

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A more specialized field of investigation, that of the carbohydrate-nitrogen ratio in plants, has been investigated primarily by horticulturists. Some of the most noteworthy work is that of Kraus and Kraybill (17), Reid (27), Harvey and Murneek (13), Hooker (14), and Murneek (23), all of whom have found that variations in the carbohydrate-nitrogen ratio are accompanied by variations in the physiological functions of the plant.

Mason and Maskell (20) studied the translocation of carbohydrates in cotton plants and found a lag in the cycle of the maximum carbohydrate movement from leaves to bark.

Janssen and Bartholomew (15, 16) found that the maximum carbohydrate production in plants growing in sand and water cultures occurred with a concentration of 3 p.p.m. of potassium in the solution, but could not obtain consistent correlations in carbohydrate production by the same kind of plants grown on field soils fertilized with different amounts of potassium fertilizer.

Clements (6) found reciprocal relations for nitrogen and carbohydrates in field peas grown in water cultures, and Woo (32) obtained similar results with certain plants grown in the field. Hartwell (12) found that a number of factors associated with retarded growth caused an increase in the starch content of potato vines.

EXPERIMENTAL OUTLINE

Two tiers of fertilized plots were used, each plot being 2 rods by 4 rods, with a check strip 1 rod wide between the two tiers of plots. The following fertilizers were used: 4–16–0, 0–16–8, 4–16–8, and 4–16–12. Each treatment was replicated four times. Samples for analysis were taken from one set of the replications and the remaining three sets were used for obtaining the crop yields. No samples were taken from the 4–16–12 plot, because in some previous work it was found that the yields were almost the same on the plots treated with the 4–16–8 and 4–16–12 fertilizer. The latter treatment was included in the experiment in order further to compare it with the 4–16–8 treatment from the standpoint of its effects on the crop yields.

The experiment field was located about 1½ miles from the laboratory, where the analyses were made. The soil of the experiment field was Hillsdale sandy loam. This soil type is one of the principle moranic soils of south central Michigan, and is well adapted to the growing of potatoes. The fertilizers applied were made from ammonium sulfate, 40 per cent superphosphate, and muriate of potash. They were broadcast by hand at the rate of 750 pounds an acre and worked into the soil with a spring-tooth drag shortly before the potatoes were planted.

WEATHER CONDITIONS

As the season of 1930 was extremely dry, the yield of the potatoes ranged only from 90 to 100 bushels an acre, and the plots showed no differences in yields attributable to the fertilizer treatments. In the years of normal rainfall, however, yields of from 200 to 300 bushels an acre were obtained.

The plots were sampled on July 23, August 12, and September 4. During this period only 0.58 of an inch of rain fell, although the normal rainfall for the period is from 4 to 5 inches. The first samples were taken when the blossoms were forming on the plants, the second as the plants began to set tubers, and the third, when the plants were in the last stages of maturity.

An attempt was made to take samples only on a clear day and they were never taken unless most of the day previous had been clear. This precaution was taken in order that the normal production and utilization of carbohydrates would be occurring within the plant at the time samples were taken. The data in table 1, from the United States Weather Bureau at East Lansing, give the meteorological conditions on the dates of and for two days previous to each sampling.

Regardless of the dry weather, the plants appeared perfectly normal when the first and second samples were taken, although at the time of the first sampling, the plants in the fertilized plots were much larger than those in the check rows. This difference was not so noticeable when the second samples were taken, showing that the larger plants were not growing so rapidly as the smaller ones. On September 4, when the third samples were taken, the plants showed clearly that they were injured by the prolonged drought. The leaves were

TABLE 1

Meteorological conditions on sampling dates and two days previous to each sampling

DATE	MINIMUM TEMPERATURE	MAXIMUM TEMPERATURE	CHARACTER OF DAY	SUNSHINE	PRECIPITATION
	°F.	°F.		*per cent*	*inches*
July 21	67	89	Partly cloudy	68	0.04
July 22	63	82	Partly cloudy	72	0
July 23	58	83	Clear	99	0
August 10	52	73	Clear	96	0
August 11	45	73	Clear	94	0
August 12	45	73	Clear	100	0
September 2	68	87	Cloudy	23	0.07
September 3	54	82	Clear	95	0
September 4	46	79	Clear	100	0

curled, and the plants looked slightly wilted from the middle of the day until after nightfall, although they were turgid in the morning. No differences could be seen between the size of plants in the check rows and the plants on the fertilized plots.

METHODS OF SAMPLING

At each date of sampling, seven sets of samples were taken. Starting at midnight, samplings were made every four hours until midnight of the following day. In taking the samples, the plants from the whole hill were selected, and the tops cut off about 2 inches above the ground. A sufficient number of hills were taken to fill a 20-pound capacity paper bag with the plants. In the first sampling, the tops from six hills were taken from each fertilized plot, but the plants from eight hills were required from the check plot to give sufficient material for study. Plants from four hills of each fertilized plot and five hills of the check plot gave sufficient material in the second sampling, whereas

the plants from four hills, irrespective of treatment, gave sufficient material in the third sampling.

PREPARATION OF SAMPLES

The samples were rushed to the laboratory immediately after cutting, and were separated into two portions—leaves and stalks. A part of each portion was then chopped to pieces with a paper cutter and 50 gm. of each portion was put into separate pint Mason jars, each containing 400 cc. of boiling alcohol to which had been added a little calcium carbonate to neutralize any acids that might be present in the plants. The jars were immediately set on the steam bath to boil for 1 hour in order to kill the enzymes, and thus prevent changes in the carbohydrates present in the plant tissue. The remainder of each sample of leaves and stalks was ground separately in a food chopper and from 100 gm. of the ground tissue the juice was expressed according to the method described by Sayre and Morris (29). The amount of expressed juice from the leaf samples did not in any one case exceed 50 cc. Each lot was measured, however, and poured into bottles containing 200 cc. of boiling alcohol which contained small quantities of calcium carbonate to keep the juice alkaline. The juice from 100 gm. of ground stalks usually amounted to about 60 or 70 cc., 50 cc. of which were pipetted off and preserved in the same manner as the juice from the leaf samples. The samples thus obtained were also put on the steam bath and boiled for one hour. All of the samples of both tissue and juice were tightly sealed in the containers after boiling, and stored to be analyzed later.

METHODS OF ANALYSIS

The samples that were preserved in alcohol were filtered, washed with 80 per cent alcohol, and made up to a volume of 1,000 cc. for tissue samples, and 200 cc. for juice samples. From these quantities aliquot portions were taken and alcohol was removed by distillation under reduced pressure, Claissen flasks being used. The solutions, when reduced to about 30 cc. were washed from the Claissen flasks into 100-cc. volumetric flasks with hot water and cooled down rapidly to room temperature. They were then made up to volume, mixed well, and the contents poured into dry 100-cc. centrifuge tubes containing 0.5 gm. of neutral lead acetate. The solutions thus treated were allowed to stand for 15 or 20 minutes to permit all of the colloidal material to be precipitated, after which they were centrifuged. The supernatant liquids were poured off into other dry centrifuge tubes and powdered sodium oxalate was added to precipitate all of the lead. A second centrifuging settled the lead oxalate precipitates, and the supernatant liquids were decanted into dry beakers. Aliquot parts of these were made up to 100 cc. volume, the size of the aliquot parts depending upon the amount of sugar in the samples.

The solutions thus prepared were thoroughly mixed, 50 cc. of each put into 300-cc. Erlenmeyer flasks for the reducing-sugar determinations, and the

remaining 50-cc. portions put into another set of Erlenmeyer flasks containing 5 gm. each of citric acid crystals to hydrolyze the sucrose. The flasks containing the samples for the sucrose determinations were covered with beakers, put on a gas hot plate, and boiled from 10 to 15 minutes to complete the hydrolysis. The citric acid was then neutralized with concentrated sodium hydroxide, phenolphthalein being used as the indicator.

The reducing power of the glucose and hydrolyzed non-reducing sugars was determined by the Shaffer and Hartman (30) method, a standard solution of sodium thio-sulfate being used to titrate the excess iodine set free from a known amount of standard potassium iodide-iodate solution which was added to the reduced copper solutions. Immediately after the iodide-iodate solution was added, 12 cc. of 8 *N* sulfuric acid was added to the solution, the flask shaken to dissolve the cuprous oxide completely, then 20 cc. of a saturated potassium oxalate solution was added and the contents of the flask were titrated with standard sodium thiosulfate solution. When the end-point of the titration was nearly reached, 5 cc. of starch solution was introduced as an indicator and the titration completed.

Because of the buffer effect of the citrate ion in the sucrose samples, it was necessary to add an additional 5 cc. of sulfuric acid in the titration to bring the samples to the necessary pH.

The committee on methods for the Society of Plant Physiology (19) objects to the use of the Shaffer and Hartman method on the ground that for various plant tissues, it is not always possible to obtain a sharp end-point in the final titration. In this work, by using the recommended 15 cc. of 5 *N* sulfuric acid, the end-point was not always sharply defined, but by increasing the strength of the acid, a clearly defined end-point could always be obtained. It appears, at least with the plant tissue used in this work, that there is a rather narrow range of pH in which a sharp end-point can be obtained when this method is used. If an excess of acid is added, a white precipitate forms in the solution and the resulting titration values are too low, whereas if the pH is too high, the end-point is indistinct and the results are unreliable. The satisfactory titration after the addition of extra acid, in the case of sucrose hydrolyzed by citric acid, to correct for the buffer effect of the citrate ion, indicates the necessity of having the proper degree of acidity in the solutions. With a little practice, one can usually tell by the color of the solutions as the acid is being added just how much to use, and if one is working with tissue from only one kind of plant, the correct acidity can soon be found and no further difficulty will be encountered.

If the difficulty lies in obtaining a definite pH value in the solutions before titrating, it is clear why the tissue of different plants cannot be handled alike, because different tissues may exert a different buffer effect on the solutions, which would necessarily have to be corrected before sharp end-points could be obtained.

There are a number of other methods of determining the amount of reduced

copper (11, 19), but the Shaffer and Hartman method has a distinct advantage over most of them in that it is not necessary in this method to filter off the products of the reaction before the titration can be made for the determination of reduced copper. An excellent review of the literature on methods is given in the latter reference.

Although the method of reducing the copper described by Quisumbing and Thomas (26) was employed in this work, because it was more convenient than the Munson and Walker method (22) in handling a number of samples at one time, the Munson and Walker tables were employed for calculating the amount

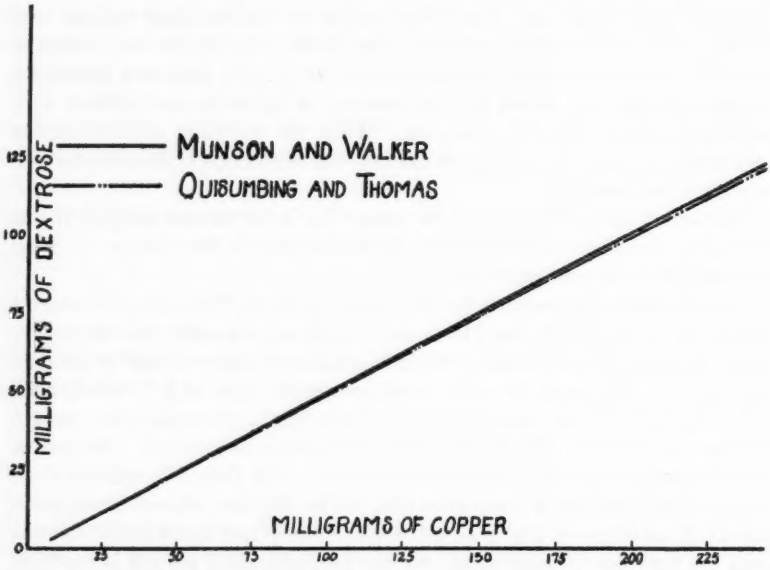

FIG. 1. A COMPARISON OF THE MUNSON AND WALKER AND THE QUISUMBING AND THOMAS TABLES FOR CALCULATING THE QUANTITY OF SUGAR FROM THE AMOUNT OF COPPER REDUCED

of sugar from the amount of copper reduced, because they are much more inclusive. The curves in figure 1 show the variations between the two tables within the range of copper used in this work. The difference between the two is so slight that the error due to interpolation in using the less complete tables of Quisumbing and Thomas would be greater in many cases than the difference between the two tables.

It was desirable to have a plan whereby the set of eight samples at each cutting, could be analyzed in one day in order to treat all samples as nearly alike as possible even with respect to the time between starting a determination and the final titration. In order to attain this end, it was necessary to use certain

procedures, such as distillation under reduced pressure to remove the excess alcohol, and the use of the citric acid hydrolysis for sucrose determinations, because of their greater speed and adaptation to the scheme of analysis. Using the centrifuge to settle precipitates is much more rapid and convenient than filtering. Careful tests of these procedures showed that accuracy was not sacrificed for speed.

EXPERIMENTAL RESULTS

The data presented in this paper are all expressed in per cent based on the green weight. All free-reducing sugars are reported as glucose and all material hydrolyzed with 10 per cent citric acid as sucrose. Total sugars are the sum of the two.

The diurnal variations of the sugars for the first sampling are shown in figures 2 and 3. The curves for the various treatments do not parallel one another as well in figure 2 as they do in figure 3. This shows that there were more variations in the glucose than in the sucrose throughout the day. Glucose variations showed rather well-defined minimums at 4 a.m. The maximums are not so marked but in general they occurred either at noon or 4 p.m. Sucrose, on the other hand, seemed to maintain a more constant level, showing neither a well-defined minimum or maximum. In practically all cases, the amounts of glucose were in excess of sucrose.

Figures giving diurnal variations for the second and third samplings are omitted because in general they are similar to figures 2 and 3. The percentages of glucose and sucrose are both somewhat higher in the later samplings, and the curves are a little more irregular.

The mode in which sucrose and glucose concentrations varied throughout the day, as reported in this paper, supports the work of Davis and Sawyer (9), who studied carbohydrate variations in the leaves and stalks of the potato plants, and Miller (21), who investigated the carbohydrate relations in the leaves of corn and sorghums. These investigators found glucose to have a minimum early in the day and a maximum in the early afternoon, whereas sucrose had a tendency to maintain an almost constant level throughout the day. They found sucrose, however, to be in excess of glucose in practically all cases, whereas the data herewith reported, with few exceptions, showed glucose to be higher than sucrose. The results reported in this work are in accord with those of Clements (7), who found that glucose was much higher than sucrose in the leaves of potatoes, sunflowers, and soybeans for the early stages of growth. Throughout the later periods of growth, the differences noted by him were not so great, but in general, the amount of glucose was in excess.

The averages of the seven samples taken at 4-hour intervals on each date of sampling, are given as daily averages in tables 2, 3, and 4. Table 2 is arranged to show the effect of fertilizer treatment on the concentrations of glucose and sucrose in the various parts of the plant.

A comparison of the averages (shown as the sum of the averages in table 2) for all samplings of leaf tissue, points out that the 0–16–8 treatment gave the lowest concentrations of sugars, whereas the check and the 4–16–8 gave the highest. In the leaf sap, the 0–16–8 continued to average lowest, with the 4–16–8 highest and the check and 4–16–0 intermediate. In the stalk tissue the check showed the highest average, with 4–16–0 and 4–16–8 about equal to

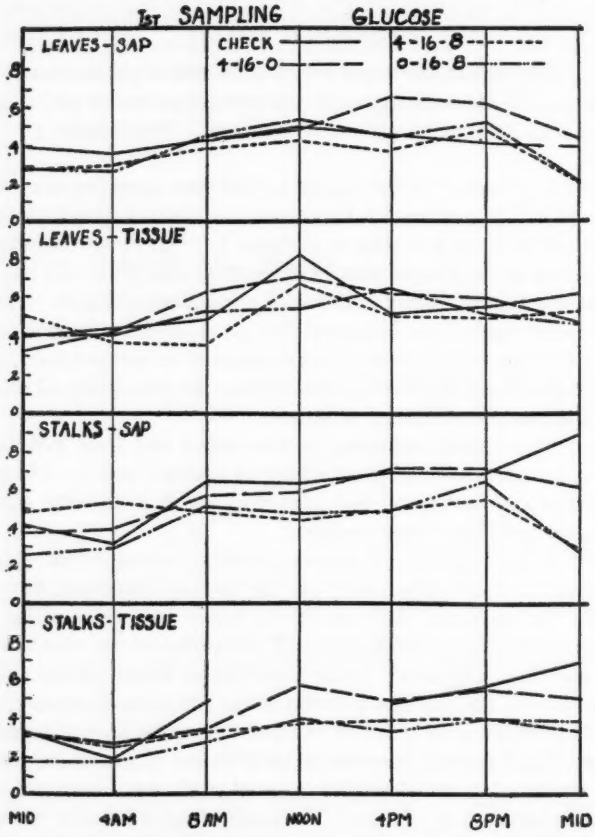

FIG. 2. RESULTS OF FERTILIZER TREATMENT ON PER CENT OF GLUCOSE IN SAMPLES TAKEN JULY 23, 1930

each other and about midway between the check and 0–16–8 averages. In the stalk sap the check and 4–16–0 were considerably higher than the other two with the 0–16–8 still averaging lowest.

The general summary, shown as the mean in the last line running from left to right in table 2, gives the average of the sum of averages. It points out that for both tissue and sap, the check has the highest average sugar content and

the 0–16–8 the lowest, with 4–16–0 and 4–16–8 about equal to each other and about midway between the extremes. These differences are not significant, showing that there were no differences in sugar content attributable to fertilizer treatments. A comparison of the daily averages for each sampling (table 2) indicates that the relation of the different treatments as shown by the mean was seldom the same as that found in any sampling. The same relation did

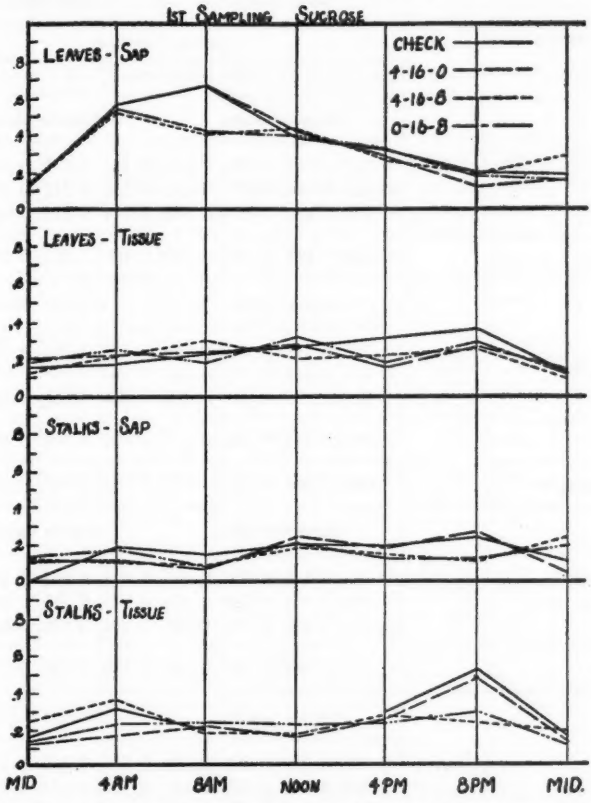

FIG. 3. RESULTS OF FERTILIZER TREATMENT ON PER CENT OF SUCROSE IN SAMPLES TAKEN JULY 23, 1930

not exist for the various treatments between determinations made from tissue and sap from the same plants, yet a comparison of the mean showed them to be very similar. Janssen and Bartholomew (16), working with plants in the field and in sand and water cultures, found no differences in carbohydrate content in plants grown in the field under varied potash treatments. When the same plants were grown in sand and water cultures, they found that concentrations of 3 p.p.m. gave maximum carbohydrate production, indicating

that under field conditions, because of so many variables, the effect of fertilizer treatments is not manifest in variations of carbohydrate content, although the treatments may greatly increase the yields.

TABLE 2

Sugars in potato plants (daily averages) as per cent of green weights

SAMPLING PERIOD	WHOLE TISSUE				EXPRESSED SAP			
	Fertilizer treatments							
	Check	4-16-0	0-16-8	4-16-8	Check	4-16-0	0-16-8	4-16-8
	Glucose—leaves				*Glucose—leaves*			
First	0.545	0.541	0.492	0.483	0.412	0.479	0.384	0.342
Second	0.606	0.624	0.582	0.634	0.535	0.512	0.583	0.600
Third	0.722	0.689	0.675	0.804	0.492	0.523	0.469	0.498
Average	0.624	0.618	0.583	0.640	0.480	0.505	0.479	0.480
	Sucrose—leaves				*Sucrose—leaves*			
First	0.233	0.217	0.213	0.202	0.348	0.293	0.305	0.324
Second	0.431	0.421	0.403	0.461	0.416	0.395	0.290	0.426
Third	0.525	0.393	0.447	0.462	0.474	0.503	0.540	0.601
Average	0.396	0.344	0.354	0.375	0.413	0.397	0.378	0.450
Sum of averages	1.020	0.962	0.937	1.015	0.893	0.902	0.857	0.930
	Glucose—stalks				*Glucose—stalks*			
First	0.456	0.434	0.305	0.343	0.613	0.563	0.419	0.467
Second	0.719	0.671	0.530	0.483	0.786	0.788	0.697	0.649
Third	0.936	0.898	0.798	0.957	0.852	0.839	0.653	0.741
Average	0.704	0.668	0.544	0.594	0.750	0.730	0.590	0.619
	Sucrose—stalks				*Sucrose—stalks*			
First	0.237	0.219	0.209	0.236	0.154	0.145	0.145	0.145
Second	0.368	0.310	0.327	0.313	0.199	0.153	0.214	0.221
Third	0.378	0.335	0.379	0.371	0.378	0.414	0.379	0.451
Average	0.328	0.288	0.305	0.307	0.244	0.237	0.246	0.272
Sum of averages	1.032	0.956	0.849	0.901	0.994	0.967	0.836	0.891
Mean	1.026	0.959	0.893	0.958	0.944	0.935	0.847	0.911

The $\frac{\text{glucose}}{\text{sucrose}}$ ratio has often been used as a means of showing carbohydrate metabolism in plants. Table 3 shows the daily averages of glucose and sucrose

for the various treatments at the different periods of sampling and gives the $\frac{\text{glucose}}{\text{sucrose}}$ ratio for each. For some of the periods of sampling the ratio was quite constant for all treatments, whereas with others, there were wide varia-

TABLE 3

Comparison of glucose and sucrose from same samples (daily averages)

FERTILIZER TREATMENT	FIRST SAMPLING			SECOND SAMPLING			THIRD SAMPLING			MEAN OF RATIO
	Glucose	Sucrose	$\frac{\text{Glucose}}{\text{Sucrose}}$	Glucose	Sucrose	$\frac{\text{Glucose}}{\text{Sucrose}}$	Glucose	Sucrose	$\frac{\text{Glucose}}{\text{Sucrose}}$	
Leaves—tissue										
Check	0.545	0.233	1.91	0.606	0.431	1.41	0.722	0.525	1.37	1.56
4–16–0	0.541	0.217	2.49	0.624	0.421	1.48	0.689	0.393	1.72	1.90
0–16–8	0.492	0.213	2.31	0.582	0.403	1.44	0.675	0.447	1.51	1.75
4–16–8	0.483	0.202	2.39	0.634	0.461	1.38	0.804	0.462	1.73	1.83
Average...	0.515	0.216	2.27	0.611	0.429	1.43	0.722	0.457	1.58	1.75
Leaves—sap										
Check	0.412	0.348	1.18	0.535	0.416	1.28	0.492	0.474	1.04	1.17
4–16–0	0.479	0.293	1.63	0.512	0.395	1.30	0.523	0.503	1.04	1.32
0–16–8	0.384	0.305	1.26	0.583	0.290	2.01	0.469	0.540	0.87	1.38
4–16–8	0.342	0.324	1.06	0.600	0.426	1.41	0.498	0.601	0.83	1.10
Average...	0.404	0.317	1.28	0.557	0.382	1.50	0.495	0.529	0.94	1.24
Stalks—tissue										
Check	0.456	0.237	1.92	0.719	0.368	1.95	0.936	0.378	2.47	2.17
4–16–0	0.434	0.219	1.98	0.671	0.310	2.16	0.898	0.335	2.68	2.27
0–16–8	0.305	0.209	1.44	0.530	0.327	1.62	0.798	0.379	2.11	1.72
4–16–8	0.343	0.236	1.45	0.483	0.313	1.54	0.957	0.371	2.58	1.86
Average...	0.384	0.225	1.69	0.601	0.329	1.82	0.897	0.366	2.46	2.00
Stalks—sap										
Check	0.613	0.154	4.00	0.786	0.199	3.95	0.852	0.378	2.25	3.40
4–16–0	0.563	0.145	3.88	0.788	0.153	5.15	0.839	0.414	2.02	3.68
0–16–8	0.419	0.145	2.89	0.697	0.214	3.26	0.653	0.379	1.72	2.62
4–16–8	0.467	0.145	3.22	0.649	0.221	2.94	0.741	0.451	1.64	2.60
Average...	0.515	0.147	3.49	0.730	0.197	3.82	0.771	0.405	1.91	3.10

tions. The ratio for the second sampling in the leaf tissue was very uniform and with one exception, was very uniform in the leaf sap. In the sap of both leaves and stalks for the third sampling, the ratio was lower for the 0–16–8 and 4–16–8 than the other two treatments. In the remaining instances the ratio

for the various treatments was very variable but there seemed to be no definite treatment that was outstandingly high or low.

The average $\frac{\text{glucose}}{\text{sucrose}}$ ratio for all four treatments was higher in the leaf

TABLE 4

Comparison of the sugars in leaves and stalks of potato plants (daily averages)

TREATMENT	FIRST SAMPLING			SECOND SAMPLING			THIRD SAMPLING			MEAN OF $\frac{\text{LEAVES}}{\text{STALKS}}$
	Leaves	Stalks	$\frac{\text{Leaves}}{\text{Stalks}}$	Leaves	Stalks	$\frac{\text{Leaves}}{\text{Stalks}}$	Leaves	Stalks	$\frac{\text{Leaves}}{\text{Stalks}}$	
Tissue–glucose										
Check	0.545	0.456	1.19	0.606	0.719	0.84	0.722	0.936	0.77	0.93
4–16–0	0.541	0.434	1.24	0.624	0.671	0.93	0.689	0.898	0.77	0.98
0–16–8	0.492	0.305	1.61	0.582	0.530	1.10	0.675	0.798	0.84	1.18
4–16–8	0.483	0.343	1.41	0.634	0.483	1.31	0.804	0.957	0.84	1.19
Average...	0.515	0.384	1.36	0.611	0.601	1.04	0.722	0.897	0.80	1.07
Tissue—sucrose										
Check	0.233	0.237	0.99	0.431	0.368	1.17	0.525	0.378	1.39	1.18
4–16–0	0.217	0.219	1.00	0.421	0.310	1.32	0.393	0.335	1.18	1.17
0–16–8	0.213	0.209	1.01	0.403	0.327	1.23	0.447	0.379	1.18	1.18
4–16–8	0.202	0.236	0.86	0.461	0.313	1.47	0.462	0.371	1.24	1.19
Average...	0.216	0.225	0.97	0.429	0.329	1.29	0.457	0.366	1.25	1.17
Sap—glucose										
Check	0.412	0.613	0.67	0.535	0.786	0.68	0.492	0.852	0.58	0.64
4–16–0	0.479	0.563	0.85	0.512	0.788	0.65	0.523	0.839	0.62	0.71
0–16–8	0.384	0.419	0.92	0.583	0.697	0.83	0.469	0.653	0.72	0.84
4–16–8	0.342	0.467	0.73	0.600	0.649	0.93	0.498	0.741	0.67	0.78
Average...	0.404	0.515	0.79	0.557	0.730	0.77	0.495	0.771	0.65	0.74
Sap—sucrose										
Check	0.348	0.154	2.26	0.416	0.199	2.09	0.474	0.378	1.25	1.87
4–16–0	0.293	0.145	2.02	0.395	0.153	2.58	0.503	0.414	1.22	1.94
0–16–8	0.305	0.145	2.10	0.290	0.214	1.35	0.540	0.379	1.42	1.62
4–16–8	0.324	0.145	2.23	0.426	0.221	1.93	0.601	0.451	1.33	1.82
Average...	0.317	0.147	2.15	0.382	0.197	1.99	0.529	0.405	1.30	1.81

tissue in the early stages of growth than in the later, whereas in the stalk tissue, the ratio increased as the plants matured. On the other hand, for the sap the ratio was highest in the second sampling, and lowest in the third for both leaves and stalks.

The mean of the $\frac{\text{glucose}}{\text{sucrose}}$ ratio for all three samplings is shown in the column at the extreme right in table 3. This mean ratio showed the 4–16–0 to have the highest ratio in three of the four instances, whereas each of the other three treatments is lowest once and the 0–16–8 and 4–16–8 showed the lowest average in the fourth case. The ratio is somewhat lower for the leaves than for the stalks. In general, the $\frac{\text{glucose}}{\text{sucrose}}$ ratio of the daily averages showed that no treatment is significantly high or low.

In order to determine whether there was any difference in the rate of translocation of carbohydrates, the concentrations of sugars in the leaves are compared with those of the stalks. The comparisons are made on the daily averages and expressed as the $\frac{\text{leaves}}{\text{stalks}}$ ratio, as shown in table 4. In general, in the tissue there was more glucose in the leaves than in the stalks of the first sampling, and less in the third, whereas in the second the check and 4–16–0 had less and the 0–16–8 and 4–16–8 had more glucose in the leaves than in the stalks. The average of the ratios for all three periods decreased with the age of the plants. The mean of the ratios of all three periods showed the check and 4–16–0 to be just slightly less than unity with the other two treatments considerably higher.

The ratios in the sap are not the same as those of the tissue. For glycose, the average of all treatments showed a ratio of less than 1 for all three samplings, whereas the ratio for sucrose is greater than 1. For both glucose and sucrose the ratio is highest in the first sampling and lowest in the third.

DISCUSSION

From the experimental results presented, no differences in sugar content, attributable to variations in fertilizer treatment were obtained, and yet rather wide differences in the per cent composition at any one time, as shown in figure 2, were often observed. Similar irregularities are reported by Clements, Miller and others. There seem to be three possible reasons for such inconsistent variations.

First, the carbohydrate content is much more affected by other factors not under control in this experiment than by variations in fertilizer treatment. This possibility is supported by the work of Arthur, Guthrie, and Newell (1), who grew a number of plants under very carefully controlled conditions, such as control of temperature, humidity, moisture, light, and plant nutrients. They found that the intensity of, and time of exposure, to light were much more important factors in carbohydrate formation than were the fertilizers which were used. In this present work, no attempt was made to measure the intensity and time of exposure to light on the various plots, which were all located within a radius of 4 to 5 rods. It was assumed that for such a small area there would be no variation in atmospheric conditions of sufficient magnitude to bring about any differences in sugar content of the plants.

Second, the method of analysis is not sufficiently accurate to give consistent results. This possibility seems rather remote because very careful tests were made on the alcoholic extracts of both tissue and sap as well as on pure solutions of glucose and sucrose by a number of methods and in all cases accurate checks were obtained.

Third, more plausible explanation than the other two, the analysis of the reducing power does not give the true relation of the various sugars as they existed in the plants at the time of sampling. Webster (31) found that the nitrate nitrogen content of alcoholic extracts decreases upon standing, and suggested that sugars might vary in a like manner. Results obtained in preliminary studies to this work, however, indicated that the composition of glucose and sucrose does not change in alcoholic extracts upon standing for at least 6 months. The change appears to have taken place between the time the samples were taken in the field and the time when all enzymes had been killed by boiling in alcohol for 1 hour. Before accurate results can be obtained in this type of investigation, it is necessary to perfect a method of sampling and preparing fresh plant material for analysis that will assure the investigator that the material he analyzes is really showing the carbohydrate relations as they existed in the plant at the time the samples were taken.

SUMMARY

Analyses showing diurnal and seasonal variations in the sugar content of both the sap and tissue of the potato plant are presented.

The percentage of glucose is in excess of sucrose in practically all samples of both leaves and stalks.

The diurnal variation of glucose showed a more or less definite cycle, with minimum concentrations usually occurring at 4 or 8 a.m., and the maximums either at noon or 4 p.m., whereas sucrose remained about constant throughout the day.

The amounts of total sugar ran higher as the plants got older.

Under the conditions of this experiment, no differences were seen in sugar content attributable to the various fertilizer treatments.

An opinion as to why carbohydrate analyses show such irregularity of variation in this and other work is presented.

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THE USE OF THE MOISTURE EQUIVALENT IN THE TEXTURAL CLASSIFICATION OF SOILS

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Some form of mechanical analysis has been used in this country for many years as a means for assigning textural class names to soils encountered during soil surveys. The function of a textural name is considered to be that of conveying an approximate idea of the size distribution curve of a soil. In this case it is apparent that the mechanical analysis, if such can be performed in a manner providing results of fundamental significance, is the most reasonable basis for such a classification. When used in this capacity the individual soil samples dealt with are usually part of a succession of depth samples collected from a single soil profile which is considered to be representative, as judged by field observation, of the entire area covered by a single soil type.

In addition, the textural name is widely used by laboratory and field workers to convey some idea of the field character and behavior of the soil. Any method of mechanical analysis which aims at complete dispersion necessarily includes in its early stages the partial or entire reconstruction of the system colloid-absorbed cations. However desirable this may be from the viewpoint of the investigator in other respects, it gives a more or less imperfect picture of the physical properties of the soil as it exists in the field. The ideas conveyed by this statement have been dealt with by Keen (9, 10) and will not be further enlarged upon. Although the mechanical analysis of a soil sample after a high degree of dispersion may be regarded at present as the best, and the least arbitrary, reference point for the expression of its texture, and although it constitutes a valuable method of approach for certain kinds of research in soil physics, it is well known that a gap exists between the mechanical analysis and its interpretation in terms of field behavior. Zunker (18, 19) has made extensive observations in this direction, having examined the relations existing between the specific surface and certain field characteristics of soils.

A question arises, therefore, concerning the advisability of making expensive analyses of single soil samples, which at best are slightly variable from place to place, for which the immediate need is some basis for textural classification and an indication of probable field behavior. Various single-valued expressions have been used for the purpose of reducing the time involved in mechanical analyses and the unwieldiness of the results obtained. No application of any single-valued expression to textural classification appears to have

been made, however, although various equations have been proposed to express the approximate relation existing between certain of these and the corresponding mechanical analyses of soil samples.

The moisture equivalent was originally proposed by Briggs and McLane in 1907 as a purely arbitrary single-valued expression of water retentive power and was later mentioned by them as a possible basis for soil classification (4). The connections between moisture equivalent and water retaining capacity of soil columns both in field and laboratory have since received much attention, and useful relationships have been established (2, 7, 13, 17). For this reason the moisture equivalent has much interest under the agricultural conditions of western North America and wherever moisture may be the limiting factor in production. It has also been shown to bear a general, though not exact, relation to the permanent wilting percentage of soils (5, 1, 16) and to the non-sticky plastic range (3).

The following equations have been proposed to connect the mechanical analysis of a soil and its moisture equivalent:

Briggs and Shantz, 1912 (4):
M.E. = 0.02 sand + 0.22 silt + 1.05 clay
Alway and Russel, 1916 (1):
M.E. = 0.14 sand + 0.27 silt + 0.53 clay
Smith, 1917 (14):
M.E. = 0.023 sand + 0.25 silt + 0.61 clay
Middleton, 1920 (11):
M.E. = 0.063 sand + 0.291 silt + 0.42 clay

Smith's equation is the result of direct determinations of the moisture equivalents of soil separates obtained from several Californian soil types of varying texture. The others were calculated by the method of least squares. In all cases the terms "sand," "silt," and "clay" refer to the U. S. Bureau of Chemistry and Soils classification for analyses made by the old centrifugal method following simple pretreatment by shaking with ammonia water only. It is uncertain to what extent these equations would be changed if the mechanical analyses were to be made by methods producing a higher state of dispersion than was obtained by the ammonia pretreatment and centrifugal separation. In a large number of cases the clay content might be expected to be increased at the expense of the silt, thus changing the relative weights attributable to these two components in the calculation of the moisture equivalent. Thomas and Harris (15) and Joseph (8) have examined the effect of the constitution of the clay and silt particles upon the moisture equivalent. It is evident from their results that the chemical composition of the particle itself and of its absorbed cations may influence the moisture equivalent value considerably.

The additive nature of the effect upon the moisture equivalent of the physical components of the soil has been investigated further in this laboratory by the direct method of measuring the moisture equivalents of mixtures of soil of known moisture equivalent values. The results so obtained led to a considera-

tion of the utility of a combined moisture equivalent-soil class diagram and the application of the moisture equivalent for purposes of textural classification. The method and results follow.

MOISTURE EQUIVALENTS OF SOIL MIXTURES

Procedure

Five samples of soil and a quartz sand were used in the experiments. Both soils and sand were air-dry. The finer materials contained many hard lumps which necessitated the use of a rubber-tipped pestle and mortar, and in the case of the Sacramento clay a hard-tipped pestle, to reduce them to a condition fine enough to pass through a 1-mm. brass sieve. A 1-mm. rather than a 2-mm. sieve was used to make possible the preparation of more nearly homogeneous mixtures of soil. The samples were thoroughly mixed, placed in glass jars, and sealed, after which their contents of moisture were determined. From the

TABLE 1

Moisture equivalents of the soils used in preparation of the mixtures

NUMBER	NAME	MOISTURE EQUIVALENT
		per cent
24	Quartz sand	0.34
13	Gravelly sand	4.4
14	Yolo sandy loam	14.3
15	Yolo sandy loam	21.1
22	Yolo loam	24.4
30	Sacramento clay	36.2

moisture content of each a table was prepared, giving the different weights of each air-dry sample which it would be necessary to take in preparing a series of mixtures with a second sample in which the two samples would be present in certain proportions on the dry basis. For every pair of the six samples, seven mixtures were prepared, such that one member of every pair was present as 12.5, 25, 37.5, 50, 62.5, 75, and 87.5 per cent, dry basis, of the mixture. Sufficient material was taken in the preparation of each mixture to give 90 gm. of mixture, the mixing being done as thoroughly as possible on a sheet of paper with a spatula. The six samples provided 15 possible combinations, each combination being used in the seven different proportions already mentioned. The moisture equivalent determinations of the original samples and of their mixtures were made in duplicate by the usual method of procedure used at this station, the speed of the centrifuge being checked by four speed measurements made with a Peerboom and Schurmann tachometer at 10-minute intervals during each run. The moisture equivalents were expressed as percentages of the dry weight of the material. In case of deviations between duplicates in excess of 2 per cent of their average moisture equivalent, the

TABLE 2

Moisture equivalents of soil mixtures

COMBINATION NO.	SOILS USED IN COMBINATION*		m		PERCENTAGE (x) OF SOIL *b* PRESENT IN MIXTURE									AVERAGE DEVIATION
	Soil *a*	Soil *b*			0	12.5	25.0	37.6	50.0	62.5	75.0	87.5	100	
					per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	
A	15	22	0.033	Calculated M.E.†	*21.1*	*21.5*	*21.9*	*22.3*	*22.8*	*23.2*	*23.6*	*24.0*	*24.4*	
				Observed M.E.	21.1	21.8	21.9	22.4	22.7	23.0	23.9	24.2	24.4	
				Difference	....	+0.3	0	+0.1	−0.1	−0.2	+0.3	+0.2	....	±0.17
B	24	13	0.041	Calculated M.E.	*0.3*	*0.8*	*1.3*	*1.8*	*2.4*	*2.9*	*3.4*	*3.9*	*4.4*	
				Observed M.E.	0.3	1.0	1.0	1.8	2.4	2.8	3.7	3.9	4.4	
				Difference	....	+0.2	−0.3	0	0	−0.1	+0.3	0	....	±0.13
C	14	15	0.068	Calculated M.E.	*14.3*	*15.2*	*16.0*	*16.9*	*17.7*	*18.6*	*19.4*	*20.4*	*21.1*	
				Observed M.E.	14.3	14.9	16.6	17.5	17.9	18.7	19.6	19.2	21.1	
				Difference	....	−0.3	+0.6	+0.6	+0.2	+0.1	+0.2	−1.2	....	±0.46
D	13	14	0.099	Calculated M.E.	*4.4*	*5.6*	*6.9*	*8.1*	*9.4*	*10.6*	*11.8*	*13.1*	*14.3*	
				Observed M.E.	4.4	5.7	7.0	8.3	9.4	11.2	11.3	13.2	14.3	
				Difference	....	+0.1	+0.1	+0.2	0	+0.6	−0.5	+0.1	....	±0.23
E	14	22	0.101	Calculated M.E.	*14.3*	*15.6*	*16.8*	*18.1*	*19.3*	*20.6*	*21.9*	*23.1*	*24.4*	
				Observed M.E.	14.3	15.8	17.3	18.2	20.0	21.6	21.9	23.4	24.4	
				Difference	....	+0.2	+0.5	+0.1	+0.7	+1.0	0	+0.3	....	±0.40
F	22	30	0.118	Calculated M.E.	*24.4*	*25.9*	*27.3*	*28.8*	*30.3*	*31.8*	*33.3*	*34.7*	*36.2*	
				Observed M.E.	24.4	25.0	26.9	28.0	29.1	31.0	33.3	34.9	36.2	
				Difference	....	−0.9	−0.4	−0.8	−1.2	−0.8	0	+0.2	....	±0.61
G	24	14	0.140	Calculated M.E.	*0.3*	*2.1*	*3.8*	*5.5*	*7.3*	*9.0*	*10.8*	*12.6*	*14.3*	
				Observed M.E.	0.3	–	4.6	5.8	7.5	9.4	10.8	12.6	14.3	
				Difference	....	–	+0.8	+0.3	+0.2	+0.4	0	0	....	±0.28

H	15	30	0.151	Calculated M.E.	*21.1*	*23.0*	*24.9*	*26.8*	*28.6*	*30.5*	*32.4*	*34.3*	*36.2*	
				Observed M.E.	21.1	23.7	25.3	26.5	28.1	29.7	32.3	34.8	36.2	
				Difference	....	+0.7	+0.4	−0.3	−0.5	−0.8	−0.1	+0.5	....	±0.47
I	13	15	0.167	Calculated M.E.	*4.4*	*6.5*	*8.6*	*10.7*	*12.8*	*14.8*	*16.9*	*19.0*	*21.1*	
				Observed M.E.	4.4	6.6	9.1	11.6	13.5	15.7	17.3	18.7	21.1	
				Difference	....	+0.1	+0.5	+0.9	+0.7	+0.9	+0.4	−0.3	....	±0.54
J	13	22	0.200	Calculated M.E.	*4.4*	*6.9*	*9.4*	*11.9*	*14.4*	*16.9*	*19.4*	*21.9*	*24.4*	
				Observed M.E.	4.4	7.4	10.5	13.3	15.9	18.0	20.4	22.6	24.4	
				Difference	....	+0.5	+1.1	+1.4	+1.5	+1.1	+1.0	+0.7	....	±1.04
K	24	15	0.208	Calculated M.E.	*0.3*	*2.9*	*5.5*	*8.1*	*10.7*	*13.3*	*15.9*	*18.5*	*21.1*	
				Observed M.E.	0.3	3.4	5.3	9.0	11.4	14.3	16.6	19.3	21.1	
				Difference	....	+0.5	−0.2	+0.9	+0.7	+1.0	+0.7	+0.8	....	±0.69
L	14	30	0.219	Calculated M.E.	*14.3*	*17.0*	*19.8*	*22.5*	*25.3*	*28.0*	*30.7*	*33.5*	*36.2*	
				Observed M.E.	14.3	17.1	20.7	23.6	25.2	28.4	31.0	35.2	36.2	
				Difference	....	+0.1	+0.9	+1.1	−0.1	+0.4	+0.3	+1.7	....	±0.66
M	24	22	0.241	Calculated M.E.	*0.3*	*3.3*	*6.3*	*9.3*	*12.4*	*15.4*	*18.4*	*21.4*	*24.4*	
				Observed M.E.	0.3	3.7	7.6	10.7	13.4	17.7	19.3	21.5	24.4	
				Difference	....	+0.4	+1.3	+1.4	+1.0	+2.3	+0.9	+0.1	....	±1.06
N	13	30	0.318	Calculated M.E.	*4.4*	*8.4*	*12.4*	*16.3*	*20.3*	*24.3*	*28.2*	*32.2*	*36.2*	
				Observed M.E.	4.4	9.2	14.0	17.9	21.5	25.3	28.9	34.0	36.2	
				Difference	....	+0.8	+1.6	+1.6	+1.2	+1.0	+0.7	+1.8	....	±1.24
O	24	30	0.359	Calculated M.E.	*0.3*	*4.8*	*9.3*	*13.8*	*18.3*	*22.7*	*27.2*	*31.7*	*36.2*	
				Observed M.E.	0.3	5.4	10.4	15.4	20.0	23.3	28.3	32.8	36.2	
				Difference	....	+0.6	+1.1	+1.6	+1.7	+0.6	+1.1	+1.1	....	±1.11

* See table 1 for names of soils corresponding to the numbers here given.

† M.E. = Moisture equivalent.

determinations were repeated for those soil mixtures having a moisture equivalent greater than 10 per cent; for the coarser textured soils wider deviations were permitted.

The series and class names of the soils represented by these samples are given in table 1. The class names are obtained from the mechanical analyses previously determined by the old centrifugal method and by reference to the triangular diagram of Davis and Bennett (6). Sample 15 is a heavy sandy loam, almost a loam. Sample 22 is a heavy loam, and as judged by its feel is gen-

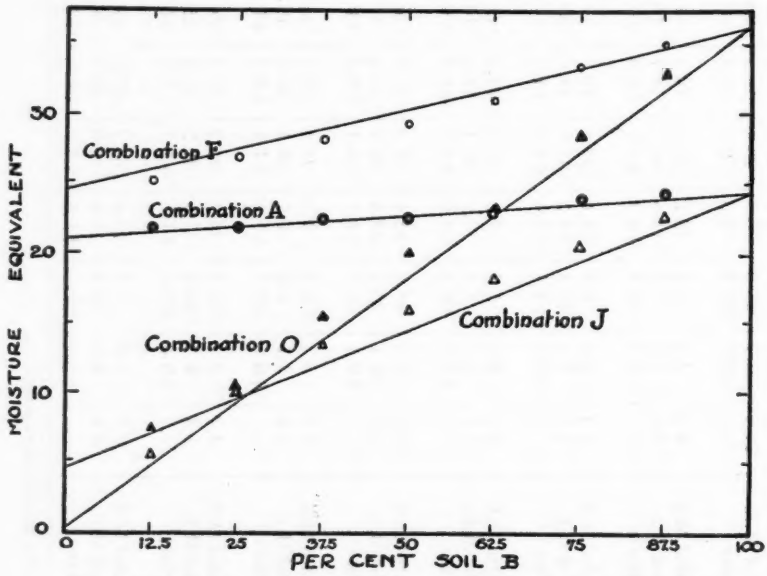

FIG. 1. CALCULATED AND OBSERVED MOISTURE EQUIVALENTS FOR FOUR SOIL COMBINATIONS

Combination A—soil 15 (M.E. = 21.1) and soil 22 (M.E. = 24.4).
Combination F—soil 22 (M.E. = 24.4) and soil 30 (M.E. = 36.2).
Combination J—soil 13 (M.E. = 4.4) and soil 22 (M.E. = 24.4).
Combination O—soil 24 (M.E. = 0.3) and soil 30 (M.E. = 36.2).

erally referred to as a clay loam. The mechanical analysis shows it to be on the boundary between a loam and a clay loam.

Table 2 contains the average results of the duplicate determinations of the moisture equivalents of the samples and their mixtures. That member of a soil combination having the lower moisture equivalent is designated *soil a*, the member having the higher moisture equivalent being termed *soil b*. The calculated and observed moisture equivalents for combinations A, F, J, and O are represented in figure 1. The observed moisture equivalent values evidently lie very close to a straight line connecting the moisture equivalents

of the component samples. Assuming an entirely additive relationship between moisture equivalent and physical components, the equation of the line representing the moisture equivalent of mixtures of soil samples of known moisture equivalent values may be written as

$$y = mx + y_a$$

where y = the moisture equivalent in per cent of a homogeneous mixture of two soils, a and b.

y_a = the moisture equivalent of the soil having the lower moisture equivalent.

y_b = the moisture equivalent of the soil having the higher moisture equivalent.

$$m = \frac{y_b - y_a}{100}.$$

and x = per cent of soil b in the mixture.

The value of y in the foregoing equation has been calculated for each mixture in the 15 combinations and the calculated values are included in table 2, in italics. The soil combinations and the resulting moisture equivalents are tabulated in order of increasing values of m, that is, in order of increasing difference between y_b and y_a. A close parallelism between observed and calculated values is evident throughout.

From the average deviations contained in the righthand column of table 2, it is evident that greater difference between observed and calculated values occurs in mixtures having widely different moisture equivalents. The average deviation for all mixtures is ± 0.61 moisture equivalent. For those mixtures in which y_b exceeded y_a by a moisture equivalent percentage of 20 or less the average deviation is only ± 0.44. The increase in the deviation which occurs with increase in m is evidently due to the difficulty of obtaining a uniform mixture of the component materials, the difficulty becoming greater with decrease in similarity of proportions of different sized particles. Other errors of operation are probably emphasized in the result when the soil samples concerned are very dissimilar.

These results suggest the direct application of Smith's values connecting the moisture equivalent and mechanical analysis to the soil texture diagram of Davis and Bennett. Suppose the equilateral triangle diagram, with its inscribed class boundaries and triangular coördinate system be considered as the base, ABC, of a truncated prism (see fig. 2), having vertical edges measuring upwards respectively 2.3 units (AA′) from the 100 per cent sand apex, 25 units (BB′) from the 100 per cent silt apex, and 61 units (CC′) from the 100 per cent clay apex. Thus a three-dimensional figure is obtained whose height at any point, corresponding to a given individual soil sample correctly placed on the plane of the inscribed base, provides the moisture equivalent of the soil sample concerned. This is simply an extension of the idea, experimentally established by the present results, that the moisture equivalent of a soil mixture is represented by some value intermediate between the moisture equivalents of the two soil components, and of a magnitude depend-

ent upon the proportions of those components present in the mixture. In figure 2 the moisture equivalent for a mixture of sand and silt containing x per cent silt is given by the vertical distance y. Similarly the height y' gives the moisture equivalent of a mixture of sands and clay represented by the point E. Soils of intermediate composition between D and E, such as F, will have moisture equivalents between y and y'. Soil F, for example, would have the moisture equivalent y''. By location along A′B′, B′C′, and A′C′ of the pos-

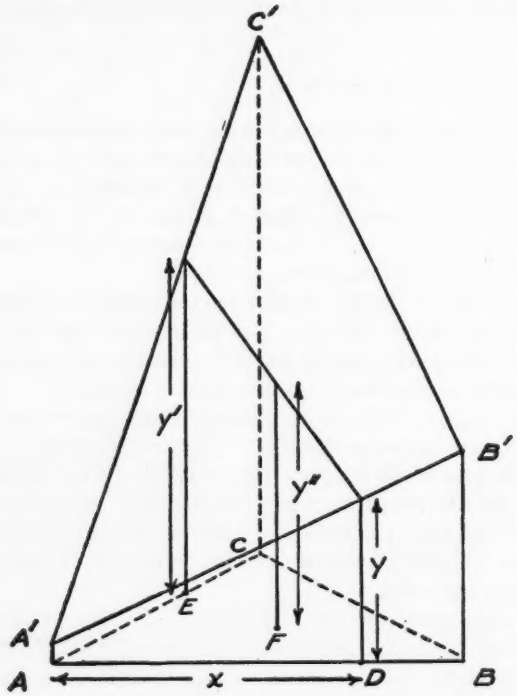

FIG. 2. DIAGRAM ILLUSTRATING RELATION BETWEEN MOISTURE EQUIVALENT AND MECHANICAL COMPOSITION

sible moisture equivalent values, points of equal height in the plane A′B′C′ may be connected by straight lines. A moisture equivalent scale is thus obtained upon the sloping area which may be projected conveniently upon the horizontal base, as in figure 3, whereby a diagram is obtained providing for the rapid, direct, graphic calculation of the moisture equivalent of a soil sample having a given textural composition. Conversely, given the moisture equivalent and the amount present of any one of sands, silt, or clay, the mechanical analysis and hence the class name may be determined graphically.

The efficiency of the method outlined as a means of obtaining the textural class name of a soil sample was tested for 169 surface and subsoil samples from three soil survey areas in California. In order to obtain in addition a more representative set of soils for consideration reference was made to the paper by Middleton (11) which includes mechanical analyses and moisture equivalents for 34 soils from various parts of the country. Textural class names were then assigned by means of a larger scale, modified form of figure 3, a moisture equivalent-sand diagram, in which percentage of sands was marked off along the horizontal axis and percentage moisture equivalent along the vertical axis. The boundary lines of the areas representing the various textural classes were then drawn in through those coördinate points representing transitions

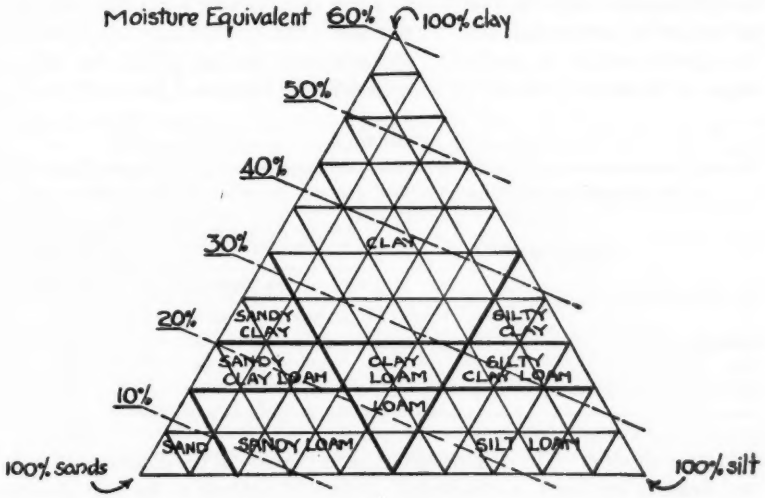

FIG. 3. SOIL CLASS-MOISTURE EQUIVALENT DIAGRAM BASED ON DAVIS' AND BENNETT'S TRIANGLE AND SMITH'S MOISTURE EQUIVALENT VALUES FOR SOIL SEPARATES

from one textural class to another. The values for sand content and moisture equivalent were obtained and the class name was decided from the area in which the points of intersection fell. Any points falling outside the diagram were assigned to the class whose area they most nearly approached. Of the Californian samples examined, 100 were analyzed by the centrifugal method of separation and 69 by the pipette method following acetic acid, hydrogen peroxide, hydrochloric acid, and sodium oxalate pretreatment (12). The results are given in table 3. "Complete agreement" signifies that the same class name would be given to the soil sample whether the name were determined by means of the triangular mechanical analysis class diagram of Davis and Bennett, or by the moisture equivalent-sand diagram. When the two methods

of obtaining the texture name resulted in the names of adjacent class areas being assigned to a single sample the agreement was considered as "fair." In some cases the soils showing "fair" agreement were actually very near to complete agreement, and separated from each other by only a short distance, yet falling in two different classes. When a greater discrepancy than the foregoing was obtained the agreement was termed "poor."

A moisture equivalent-sand diagram method of textural classification may be expected to attribute more weight to the field behavior of the soil than does the mechanical analysis, since two properties have been used which are associated closely with field methods of classification and field behavior and which may be expected considerably to influence the judgment of the field observer. This probably explains the higher degree of agreement between the mechanical analysis and moisture equivalent methods of class naming when the centrifugal method of mechanical analysis was used than was obtained by means of the pipette method of analysis. The analytical method giving the higher degree of dispersion probably overemphasized the fineness of the soil from the

TABLE 3

Degree of agreement obtained between class names determined by triangular diagram (Davis and Bennett) and moisture equivalent-sand diagram (based on Smith's values)

AGREEMENT	AMMONIA PRETREATMENT—CENTRIFUGAL SEPARATION		PEROXIDE-ACID-SODIUM OXALATE PRETREATMENT—PIPETTE SAMPLING AND SIEVING
	Californian soils	Middleton's soils	
	per cent	*per cent*	*per cent*
Complete	56	65	29
Fair	38	35	39
Poor	6	0	32

field point of view. The class designations in the case of the diagram of Davis and Bennett are based on centrifugal mechanical analysis of the soils already named texturally by experienced men who collected them (6). This diagram can hardly be expected to continue to serve as the basis of textural classification if a method of analysis is used which no longer has the field significance of that upon which the diagram itself is based.

SUMMARY

The results of moisture equivalent determinations of mixtures of soil samples of known moisture equivalent values and in known proportions are presented in support of the suggestion that a single diagram based upon Davis' and Bennett's soil class triangle, Smith's directly determined moisture equivalent values of soil separates, and the total sand content, may be used to give useful approximations to the textural class names. The texture of the sample would thus be fixed by means of moisture equivalent and total sand determinations only, both being operations which can be performed rapidly. In

addition, the highly useful moisture equivalent value would be available for various other purposes. Since the diagram of Davis and Bennett is in turn based upon the mechanical composition of the soil as analyzed by a mild form of pretreatment and centrifugal separation (the Bureau of Soils old method of analysis), it is evident that this method of analysis alone can be used for an accurate designation of the class names of normal soils where this particular triangular diagram is to be used.

The purpose of the mechanical analysis of samples collected during the course of a soil survey is primarily to guide the soil surveyors in their application of textural names and secondarily to indicate the probable field behavior of the soil. It is not to be expected, therefore, that a method of analysis, the object of which is complete dispersion of the soil, will be well fitted to the needs of a soil survey, although such a method has much to recommend it for soil physics researches and detailed profile examinations.[1]

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INVESTIGATIONS CONCERNING SEPARATION OF SIMILARLY CHARGED IONS FROM SOILS BY ELECTRODIALYSIS[1]

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In electrodialysis experiments the different cations which are liberated from the solutions or suspensions contained in the middle compartment of a three-chambered apparatus will be accumulated in the cathode chamber as hydroxides. If the material which is dialyzed contains a compound of cations that can be liberated, for example K, Na, Ca, Mg, Fe, and Al, the cathode water after some time will consist of a mixture of the respective hydroxides, of which the hydroxides of magnesium, iron, and aluminum will precipitate more or less completely, because they are relatively insoluble in alkaline solution. The formation of these hydroxides can, if more than traces are present, be distinguished since the cathode water changes from colorless to light yellow.

The anions liberated from an electrodialyzed medium migrate to the anode where a formation of acids in the anode water takes place. If we deal with soil suspensions which contain humus material the dialyzate in the anode chamber will consist of a mixture of mineral acids and electrodialyzable "humic acids." The presence of "humic acids" will make the color of the anode water more or less yellow or brown, depending on the amount which enters the anode chamber. In the electrodialysis of muck or peat soil the anode water may be dark brown.

The analytical procedure for separation of the different ions in the dialyzates obtained by electrodialysis of soils is relatively simple compared with the procedure of analysis of extracts from leaching experiments. This is because of the greater degree of purity of the dialyzates as compared to the extracts obtained by treating the soil samples with acids or solutions of neutral salts. If it were possible, however, to separate the different ions in the apparatus itself, before the dialyzates were drained from the respective chambers, it would be of great theoretical interest and also have considerable practical value, since it would simplify the analytical procedure. Such a separation of equally charged ions would find application in different branches of science, and not alone in the investigation of soils.

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The work reported in this paper was performed by the author while located in the soils department of Ohio State University, under a fellowship supported by the Rockefeller Foundation. The author gratefully acknowledges the helpful suggestions and criticisms given by Dr. R. Bradfield during the progress of the investigations.

The possibility of an electrodialytical separation of ions bearing similar electrical charge has, according to the author's knowledge, never been discussed in the literature. The idea that at least a partial separation of the ions liberated from soils could be obtained, arose in connection with a series of electrodialysis previously performed by the author (3). The fact that some of the liberated ions form, and some ions do not form, precipitates in the electrode chambers, was the fundamental principle upon which the idea of separation was based.

APPARATUS AND METHODS OF INVESTIGATION

In order to test the possibility of a separation of different cations and different anions the author has constructed a five-compartment electrodialysis apparatus, which is shown in plate 1.[3] This apparatus was developed from the author's previously described three-compartment cell (2) by inserting two new chambers between the middle chamber and the end chambers, one chamber at each side. These intermediate chambers have a capacity of 60 to 65 cc. or about half the volume of the end chambers. It is possible that the volume of the intermediate chambers may later be changed so that all chambers, except the middle chamber, will be of equal capacity. However, the intermediate chambers should be made short so that the distance between the electrodes, which are placed in the end chambers, is not greatly increased. Like the other glass parts of the apparatus, the new chambers are made of Pyrex glass.

A five-compartment apparatus, or a multiple cell with more than three compartments, can easily be constructed from the three-compartment cell. The requirements are only extra chambers and an extra set of sufficiently long rods, which are fitted to the frame of the three-compartment apparatus. As it is convenient to have supports beneath the connections of the chambers when the different parts are joined together, small supports of wood have been made. These are so formed that they fit to the flanges of the compartments which are placed together. The many connections in a multiple-compartment cell require a rather high pressure from the adjusting screw in order to get all chambers tight. Therefore all gaskets between the flanges and the cushions at the ends of the apparatus must consist of soft rubber.

A separation of different cations and different anions in the multiple apparatus has been investigated according to the following principles:

(a) By using different kinds of membranes between the different chambers.

(b) By precipitation of some of the liberated cations in the intermediate chamber (C. I) whereas other cations are allowed to migrate to the end chamber (C. II).

(a) With regard to the action and the permeability of membranes, Weiser (7) has reviewed and discussed the different theories which deal with this problem. Stated briefly, he has pointed out that separation of different substances by membranes appears to depend partly on:

[3] This apparatus can be supplied by the Central Scientific Company of Chicago.

A. The size of the pores in the membranes in relation to the size of the ions or molecules which are to be separated.

B. Solubility of the substances in the membranes.

C. Adsorption in the membranes, the latter acting as adsorbents which take up more of some substances than they do of other substances. If the solvent is adsorbed to a greater extent than the solute we have the so-called negative adsorption.

Then, the penetration of ions or molecules through membranes is more or less affected by the nature of the electrical charge on the membranes themselves, as briefly discussed in a previous publication (3).

Compared with ordinary dialysis the penetration of ions or molecules through the membranes is more or less forced because of the application of the electrical current. The case is therefore rather complicated and it is difficult to suggest before hand the types of membranes by which a separation of the similarly charged, but different ions can be obtained.

(b) The separation of different ions by precipitation of some of the ions in the intermediate chamber seems more simple when dealing with a mixture of various cations, because of the insolubility of certain hydroxides.

How far a separation of similarly charged ions can be obtained by arranging electrodes in all chambers of the multiple cell and by a regulation of the drop in potentials between the electrodes has not been investigated. By such an arrangement it would be possible to decrease the distance between the electrodes, and the process of electrodialysis would be facilitated. Then the interesting problem of separating different ions according to the size of the electrical charge on the ions could be studied.

In the following pages results are presented from a series of experiments in which an electrodialytical separation of similarly charged ions has been attempted. The experiments performed are of a preliminary character, and it was the author's intention not to present the multiple electrodialysis apparatus till more extensive investigations were made. Several circumstances, however, have made it impossible to continue at the present time and therefore it seems desirable to publish the results which already have been obtained.

EXPERIMENTAL

The experiments reported in the following paragraphs were performed with 125 volts. The distance between the electrodes, which were placed in the end chambers, varied according to the number of compartments used in the different experiments. In the three-compartment cell the electrode distance was 9 cm.; for each new compartment inserted the distance was increased by 3 cm. so that we have 12 cm., 15 cm., and 18 cm. electrode distance when four-, five-, or six-compartment cells were used, respectively. Four compartments were used when a separation of only one part of the ions liberated from the medium electrodialyzed was desired, and five compartments when a separation of both the cations and anions was the object to be attained. In a few cases an apparatus consisting of six compartments was tried.

As will be understood, the potential differences were small when four or more compartments were used, and the removal of ions was accordingly slow. In order to speed up the movement of ions the voltage should be increased, which also would increase the amperage, and when soils are to be electrodialyzed this is to be recommended.

The quantitative analyses were carried out according to standard methods: Silica was determined as SiO_2, iron and aluminum as $Fe_2O_3 + Al_2O_3$, iron alone by reduction with stannous chloride and subsequent titration with $KMnO_4$, magnesium as $Mg_2P_2O_7$, and sodium + potassium as the sum of the chlorides. The nitrate nitrogen was determined with the phenoldisulfonic acid method, phosphoric acid was determined volumetrically as ammonium-phosphomolybdate, chlorine was titrated with $AgNO_3$ solution, and sulfate was determined (approximately) with the turbidity test of the barium salt.

The qualitative tests of Fe^{+++} were made by the thiocyanate method, Fe^{++} was detected by potassium ferricyanate, aluminum with alizarin-S (sodium salt of alizarin monosulfonic acid), calcium as oxalate, magnesium as pyrophosphate, nitrate with diphenylamine, phosphate with stannous chloride in solution of ammonium molybdate, chlorine as silver chloride, and sulfate as barium sulfate. In a few samples manganese was expected to be present and tests for Mn with potassium periodate were made. [Descriptions and references to the different methods can be found in Yoe (8).]

Experiments with pure salt solutions

In order to select the types of membranes which might be suitable for use in the separation of certain ions a series of experiments were performed with pure salt solutions. Results from these experiments are given in tables 1, 2, and 3.

The experiments reported in tables 1 and 2 were made with the three-compartment cell. In table 1 are given results from experiments with solutions of ferric sulfate and calcium chloride. The purpose of these experiments was to find a membrane by which iron could be separated from calcium. The results can be summarized briefly as follows:

Fishskin, parchment, and cellophane membranes are all permeable to Ca and Fe^{+++}; rubber membrane, however, is not permeable to Fe^{+++} under ordinary experimental conditions. The rubber membrane allows penetration of Ca but the process is very slow.

When rubber was used as the cathode membrane a precipitate of iron was found on the middle chamber side of the membrane. In the other experiments iron precipitate was found in the cathode membranes themselves, but the membranes were still permeable to the ferric ion, since $Fe(OH)_3$ was detected in the cathode water. The formation of precipitate on and in the membranes must be due to hydrolysis around the membranes. This explanation is based on the statement made by Bradfield (1) that when a solution is electrodialyzed

TABLE 1

Experiments with different kinds of membranes and solutions of ferric sulfate and calcium chloride in the three-compartment apparatus

EXPERIMENT NUMBER	TIME ELECTRODIALYZED	TEMPERATURE	IONS DETECTED IN THE CATHODE CHAMBER (C.)	MEMBRANE	SOLUTIONS ELECTRODIALYZED	MEMBRANES	IONS DETECTED IN THE ANODE CHAMBER (A.)	NOTES
	hours	*°C.*						
1	2	30	Fe^{+++}	Fishskin	0.2 *N* $Fe_2(SO_4)_3$ solution	Fishskin	SO_4^{--} Fe^{+++}	In C. heavy precipitate
2	2	30	Fe^{+++}	Parchment	0.1 *N* $Fe_2(SO_4)_3$ solution	Parchment	SO_4^{--} Fe^{+++}	In C. heavy precipitate
3	2	60	Ca^{++}	Rubber	0.1 *N* $Fe_2(SO_4)_3$ and 0.05 *N* $CaCl_2$ solution	Parchment	Cl^- SO_4^{--} Fe^{+++}	Precipitate of Fe on the cathode membrane
4	2	100	Ca^{++} Fe^{+++} Fe^{++} (trace)	Parchment	0.1 *N* $Fe_2(SO_4)_3$ and 0.05 *N* $CaCl_2$ solution	Rubber	Cl^- SO_4^{--} Fe^{+++} (trace)	Precipitate of Fe on the cathode membrane
5	50	30	Ca^{++}	Rubber	0.1 *N* $Fe_2(SO_4)_3$ and 0.05 *N* $CaCl_2$ solution	Rubber	Cl^- SO_4^{--}	Very slow ion migration
6	1	40	Fe^{+++}	Cellophane	0.1 *N* $Fe_2(SO_4)_3$ solution	Cellophane	SO_4^{--} Fe^{+++}	Precipitate of Fe on the cathode membrane

TABLE 2

Experiments with different kinds of membranes and solutions of different chlorides in the three-compartment apparatus

EXPERIMENT NUMBER	TIME ELECTRODIALYZED	TEMPERATURE	IONS DETECTED IN THE CATHODE CHAMBER (C.)	MEMBRANES	SOLUTIONS ELECTRODIALYZED	MEMBRANES	IONS DETECTED IN THE ANODE CHAMBER (A.)	NOTES
	hours	*°C.*						
1	6	35	Fe^{+++}	Parchment	0.1 *N* $FeCl_3$ solution	Parchment	Cl^- Fe^{+++}	Precipitate of Fe on the cathode membrane
2	3	35	Fe^{+++}	Cellophane	0.1 *N* $FeCl_3$ solution	Cellophane	Cl^- Fe^{+++}	Heavy precipitate of Fe on the cathode membrane
3	6	35	Al^{+++}	Parchment	0.1 *N* $AlCl_3$ solution	Parchment	Cl^- Al^{+++}	Precipitate of Al on the cathode membrane
4	3	50	Al^{+++}	Cellophane	0.1 *N* $AlCl_3$ solution	Cellophane	Cl^- Al^{+++}	Heavy precipitate of Al on the cathode membrane
5	1	100	Mg^{++} (heavy precipitate)	Parchment	0.1 *N* $MgCl_2$ solution	Parchment	Cl^- Mg (trace)	Precipitate of Mg on the cathode membrane
6	1	35	Mg^{++} (heavy precipitate)	Cellophane	0.01 *N* $MgCl_2$ solution	Cellophane	Cl^- Mg (trace)	Precipitate of Mg on the cathode membrane

TABLE 3

Experiments with different kinds of membranes in the five-compartment apparatus

EXPERIMENT NUMBER	TIME ELECTRODIALYZED	TEMPERATURE	IONS DETECTED IN C. II	MEMBRANES	IONS DETECTED IN C. I	MEMBRANES	SOLUTIONS ELECTRODIALYZED (MIDDLE CHAMBER)	MEMBRANES	IONS DETECTED IN A. I	MEMBRANES	IONS DETECTED IN A. II	NOTES
	hours	*°C.*										
1	2	35	Fe^{+++}	Fish-skin	Fe^{+++}	Fish-skin	0.1 *N* $Fe_2(SO_4)_3$ solution	Fish-skin	SO_4^{--} Fe^{+++}	Fish-skin	SO_4^{--} Fe^{+++}	
2	2	100	Fe^{+++} Fe^{++} Ca^{++}	Cello-phane	Fe^{+++}	Cello-phane	0.05 *N* $Fe_2(SO_4)_3$ and 0.02 *N* $CaCl_2$ solution	Cello-phane	SO_4^{--} Fe^{+++}	Cello-phane	Cl^- SO_4^{--} Fe^{+++}	No cooling water applied
3	2	50	Na^+	Cello-phane	Fe^{+++} (NaOH added)	Cello-phane	0.05 *N* $Fe_2(SO_4)_3$ solution	Cello-phane	SO_4^{--} Fe^{+++}	Cello-phane	SO_4^{--} Fe^{+++}	In all 10 cc. of 0.2 *N* NaOH added in C. I to keep the reaction alkaline during the experiment. Portions of 2 cc. were used
4	20	35	Ca^{++} NH_4^+	Rubber	Ca^{++}	Cello-phane	0.1 *N* $CaCl_2$ and 0.1 *N* $(NH_4)_2HPO_4$ solution	Cello-phane	PO_4^{---}	Rubber	Cl^- PO_4^{---}	Precipitate of PO_4 complex on the anode rubber membrane

hydrolysis takes place, forming an alkaline reaction around the cathode membrane and an acid reaction around the anode membrane.

To the author's surprise iron was found in significant amounts in the anode fractions from all experiments where rubber was not used as anode membrane. That Fe^{+++} as an electropositive ion migrates to the anode seems very improbable, and the explanation why iron is found in the anode fractions must be that a complex ion with electronegative charge is formed. It is known that the sesquioxides of iron and aluminum are electrical ampholytes. The corresponding hydroxides are supposed to be electropositively charged, but, as Mattson (4) has pointed out, these hydroxides might under certain conditions contain more or less acid anions. If ferric hydroxide is taken as an example the $Fe(OH)_3$ molecule combines in water with the OH ion, the complex being negatively charged, and migrates toward the anode. The H ion, however, remains free and migrates to the cathode.

Now the question becomes pertinent if a formation of $Fe(OH)_3$ is probable or possible in the solutions used in the electrodialysis experiments. First it should be stated that the movement of iron toward the anode did not take place immediately when the electrical circuit was established but only after prolonged electrodialysis when a considerable amount of the acid anions was removed from the solution. If a solution of $Fe_2(SO_4)_3$ is electrodialyzed the following reactions might take place:

$$Fe_2(SO_4)_3 \rightleftharpoons 2Fe^{+++} + 3SO_4^{--}$$

$$6HOH \rightleftharpoons 6OH^- + 6H^+$$

$$2Fe(OH)_3 + 6H^+ + 3SO_4^{--} \rightarrow (4H^+ + 2SO_4^{--} \text{ removed})$$

$$\left[\begin{matrix} Fe(OH)_3 \\ Fe(OH)_3 \end{matrix}\right\rangle SO_4\Big]^{--} + 2H^+$$

The foregoing equation indicates that some of the H and SO_4 ions will be removed by electrodialysis and at a certain point of acidity in the solution the $Fe(OH)_3$ molecules formed might absorb SO_4 ions remaining in the solution. The pH value of the solution when the migration of iron toward the anode starts has not been exactly fixed, but tests with litmus paper showed that the reaction was slightly acid.

The suggested ferric-hydroxide-sulfate complex, which could be illustrated by the formula

$$\boxed{[Fe(OH)_3]_x SO_4}^-$$

must be of molecular dimensions, since it penetrates through certain types of membranes. Rubber membrane, however, seemed impermeable for this

complex, and only under extreme conditions, as when the temperature in the apparatus arose to the boiling point, which might influence the behavior of the membrane considerably, a trace of Fe^{+++} was detected in the anode water.

Experiment with a solution of ferrous sulfate (not reported in the tables) showed that iron migrates toward the anode also in the ferrous form.

A migration of iron to the anode is observed also in case a $Fe\,Cl_3$ solution is electrodialyzed, but it takes place to a less extent than when a $Fe_2(SO_4)_3$ solution is used. The reason must be that the ferric hydroxide complex combines with the divalent SO_4 ion to a greater extent than it does with the monovalent Cl ion [cf. Mattson (4)].

Solutions containing aluminum and magnesium chlorides have also been investigated in order to determine whether the aluminum and magnesium show a behavior similar to that of iron in the electrodialytical system. It was found that both the aluminum and the magnesium migrated to the anode but in the case of $MgCl_2$ solution only a trace of Mg-ions was detected in the anode fractions, whereas in the cathode fractions a heavy precipitate of $Mg(OH)_2$ was formed. This was the case regardless of whether the temperature was 35° or 100°C.

Also in the experiments with aluminum and magnesium solutions a precipitate was found on the cathode membrane. The experiments, which are reported in table 2, show that parchment and cellophane are permeable to Al^{+++} and Mg^{++}.

According to information given to the author by R. Bradfield a movement of aluminum and magnesium to the anode has also been observed in his electrodialysis experiments. The data, however, have not yet been published.

In table 3 are reported results of experiments with different kinds of membranes in the five-compartment apparatus. Experiment 1 shows that iron is found in all the side chambers when fishskin is used as membranes. The movement of iron, however, is rather slow, since a considerable amount was found in the intermediate chambers.

In experiment 2 the temperature was allowed to rise to the boiling point. Under these experimental conditions also, iron moved to the end cathode chamber together with calcium when cellophane was used as membrane between C. I and C. II. A certain amount of ferric ions was reduced to ferrous ions. A reduction of the ferric to the ferrous form was also observed in experiment 4, table 1, where the temperature reached the boiling point.

Experiment 3 differs from the others in this respect in that small portions of NaOH solution were added to the dialyzate in C. I during the time of experimentation. The purpose of such a procedure was to study whether or not it was possible to precipitate all the iron as hydroxide in C. I and prevent its movement to C. II. The reaction in C. I was observed by introducing litmus paper in this compartment. As is seen from table 3 no iron was detected in C. II in this experiment. A relatively strong alkaline reaction in the intermediate chamber should be sufficient to prevent the movement of iron to C. II,

regardless of whether or not the membrane used between C. I and C. II is permeable to iron. From experiment 2 it is seen that the transportation of calcium through C. I has not been sufficient protection to stop the iron from penetrating through the cellophane membrane to C. II, but the high temperature in that experiment might have forced the migration to a certain extent.

Experiment 4 dealt with a calcium chloride and diammonium phosphate solution. As seen from the table the rubber membrane is rather impermeable to PO_4 ion since PO_4 is detected in A. I after 20 hours of dialysis. The presence of a small amount of Ca in C. I shows that rubber also is somewhat impermeable to that ion. By repeating this experiment and electrodialysis during a period of 160 hours all PO_4 and Ca ions were removed from the intermediate chambers.

An experiment was also performed with a solution of 0.1*N* $CaCl_2$ and 0.1*N* $FeCl_3$ solution, using a six-compartment apparatus of which three compartments were placed on the cathode side and two on the anode side. The experiment was continued for 3 hours at a temperature of 30°. Parchment was used as membranes between all chambers. Qualitative tests showed that practically all the Ca was removed to the end chamber (C. III) only a trace was detected in C. I, whereas a heavy precipitate of Fe was found in this chamber. In C. II only a trace of Fe was present, but Fe was detected in significant amounts in both anode chambers.

Experiments with different types of soils

Experiment with Bedford red clay from Central College, Franklin County, Ohio, in the five-compartment apparatus. A 50-gm. soil sample which had passed through a 100-mesh sieve was used. The temperature was held at 30°C. As membrane cellophane was used between the middle compartment and the two intermediate chambers. Between the last mentioned chambers and the end chambers rubber membranes were used.

As the ammeter reading indicated that the liberation and removal of ions from the soil suspension was very slow, presumably mostly because of high resistance in the rubber membranes, dilute HCl was added in the middle chamber in order to improve conductivity. The reaction of the soil suspension, which was slightly alkaline was then changed to about pH 3. In all, 40 cc. 0.1*N* HCl were required to reach the pH value mentioned. After 48 hours of dialysis the dialyzates were drained from all chambers.

In the dialyzate from C. I a slightly white-yellow precipitate was observed, whereas the dialyzate from the end chamber (C. II) was perfectly clear and colorless. Quantitative analysis of the two fractions showed that the dialyzate from C I contained 4.35 m.e. of Mg and 0.23 m.e. of Mn per 100 gm. of soil, besides a small amount of Si but no Fe or Al.[4] Neither Ca, K, or Na was found in the dialyzate from C. I. The dialyzate from C. II contained 10.36

[4] The presence of Si in the cathode water was first observed by Odén (5) and later has been found by several investigators.

m.e. of Ca and 1.76 m.e. of K + Na per 100 gm. of soil. In this fraction no traces of Mg, Mn, Fe, Al, or Si were found.

These results seem to indicate that Mg and Mn can be separated from Ca, K, and Na under certain conditions but they do not tell whether the separation is a result of impermeability of the rubber membrane for the ions mentioned, or whether it is due to precipitation of Mg and Mn as insoluble hydroxides in the slightly alkaline solution which is formed in the intermediate chamber (C. I) as a result of the passage of Ca, K, and Na. On the contrary, the separation of Si from Ca, K, and Na might be the result of a membrane effect, since conditions in C. I should not promote a precipitation of Si in this chamber.

The anode fractions, both of which were perfectly clear and colorless, were not analyzed. It was proved, however, that the Cl ions from the added HCl moved to the end chamber (A. II) and, at least to a certain extent, were liberated as chlorine gas.

Experiment with a sample of Paulding clay, subsoil, from Paulding County Experimental Farm, Ohio. The experimental conditions varied from those described in the previous experiment in these respects: The rubber membranes were changed and instead parchment was placed between the intermediate chambers and the end chamber. No HCl was added to the soil suspension. Every 24 hours the dialyzates were drained and the chambers refilled with distilled water. The experiment was continued for 168 hours.

Qualitative determinations made in the dialyzates confirmed the fact that the cations which form strong bases move to the electrode chamber (C. II), and that the cations which form insoluble hydroxides will at least to a large extent precipitate in the intermediate chamber regardless of whether or not the membrane which is placed between these chambers is permeable to these ions. However, if the intermediate chamber is drained while the transportation of ions is proceeding, small amounts of certain ions which otherwise would be found in the end chamber might be found in the intermediate chamber. This was proved in this experiment, as the first fraction of the dialyzate from C. I contained a small quantity of Ca.

The anode fractions were tested for phosphoric acid colorimetrically. It is of interest to note that none of the fractions from A.I contained PO_4 ion. The first fraction from A. II was found to be high in phosphoric acid content; on the contrary the PO_4 in the second fraction was very low. In the third and fourth fractions the PO_4 content was medium and then it gradually decreased from fraction to fraction and by the end of 168 hours of dialysis only traces were found.

The pH value of the original soil suspension was 6.60. At the end of the second interval, which introduced the second maximum of PO_4 liberation, the pH value of the suspension was 5.35, and by the end of the fourth interval it was 4.95. In the liberation of cations corresponding increases in the amounts removed from the soil during the third and fourth intervals were found.

Experiment with a sample of Saw grass muck soil from the Everglades Experiment Station, Belle Glade, Florida.[5] The experiment was performed under almost the same conditions as those described in experiment 1. The size of the sample used for electrodialysis, however, was 25 gm., and dilute HCl was not added during the first interval of 48 hours (a). The membrane arrangement is shown in table 4. The pH value of the soil suspension was 5.65 at the start and 5.25 at the end of the first interval. After the dialyzates had been changed the experiment was continued for a new period of 48 hours during which a total of 30 cc. of 0.1*N* HCl was added in several portions (b). The pH of the suspension was then changed to about 5. The main purpose of this experiment was to determine whether or not a separation of the electrodialyzable humus acids from the mineral acids could be made.

The anode fractions from A. II were perfectly clear and colorless, whereas the a-fraction from A. I was clear but yellow and the b-fraction from the same chamber was clear but dark brown. The rubber membrane placed between A. I and A. II had evidently caused the "humic acids" liberated from the suspension in the middle chamber to be retained in A. I.

The analytical data from the experiment are given in table 4. It is of interest to note that all strong mineral acids are found in A. II. The phosphoric acid, however, is found in both A. I and A. II, but in greatest amounts in A. I. Rubber membrane is, in other words, permeable to PO_4 ions to a certain extent, as also was found in the experiment with diammonium phosphate solution (table 3, experiment 4).

In all anode fractions traces of silica were detected. According to this, rubber is not entirely impermeable to silica, at least in an acid medium. No traces of Fe + Al were found in the anode fractions.

Comparisons between the sums of mineral acids, in terms of milligram equivalents, found by chemical analysis and the amounts of acids found by titration show that there is a significant difference in the a-fraction from A. I and a rather great difference in the b-fraction from the same chamber. These differences must presumably be "humic acids" which are held back in A. I by the rubber membrane together with phosphoric acid. Similar comparisons made for the anode fractions from A. II show that the amounts found by analysis and by titration agree very well with each other.

It should be stated that the titrations of the A. I-samples were made on aliquots sufficiently diluted so that the color due to the "humic acids" did not interfere seriously with the color of the indicator used (methyl red).

In order to compare the amounts of organic material in soluble form in the different anode fractions aliquots were treated with dilute sulfuric acid and then titrated with $KMnO_4$ solution. The amounts of permanganate solution used for oxidation of the organic material should indicate whether or not the separation of the electrodialyzable "humic acids" from the mineral acids, except

[5] The author gratefully acknowledges the coöperation of Mr. J. A. Naftell who furnished this sample.

TABLE 4

Experiment with 25 gm. of a muck soil electrodialyzed in the five-compartment apparatus

FRACTIONS	M.E. IONS IN CATHODE CHAMBERS				MIDDLE CHAMBER	M.E. IONS IN ANODE CHAMBERS			
	C. II	Membrane	C. I	Membrane		Membrane	A. I	Membrane	A. II
a	No Fe + Al Ca = 2.707 Mg = 0.099 K + Na = 0.301 Sum = 3.107 Titratable bases = 3.277 Si = 0.799	Parchment	No Fe + Al Ca = 0.307 Mg = 0.119 No K + Na Sum = 0.426 Titratable bases = 0.229 Si = 0.899	Parchment	25 gm. of muck soil suspended in 450 cc. volume	Cellophane	Si (trace) PO_4 = 0.021 No NO_3 No SO_4 No Cl Sum = 0.021 Titratable acids = 0.252	Rubber	Si (trace) PO_4 (trace) NO_3 = 0.388 SO_4 = 0.300 Cl = 0.145 Sum = 0.833 Titratable acids = 0.890
b	No Fe + Al Ca = 6.617 Mg = 0.119 K + Na = 0.421 Sum = 7.157 Titratable bases = 7.012 Si = 0.799		No Fe + Al Ca = 0.639 Mg = 0.118 K + Na (trace) Sum = 0.757 Titratable bases = 0.508 Si = 0.166		In all 30 cc. of N/10 HCl added to the soil suspension		Si (trace) PO_4 = 0.106 No NO_3 NO SO_4 No Cl Sum = 0.106 Titratable acids = 0.804		Si (trace) PO_4 (small amount) NO_3 = 0.228 SO_4 = 0.080 Cl = 1.074 Sum = 1.382 Titratable acids = 1.513

phosphoric acids, is complete. It should be pointed out that no ferrous salts or other reducing substances which could interfere with the results were present in the anode fractions. In the following collocation the results are given in cc. 0.05*N* $KMnO_4$ solution per fraction:

The a-fractions: A. I used 7.2 cc. and A. II used 0.0 cc. $KMnO_4$
The b-fractions: A. I used 46.6 cc. and A. II used 0.0 cc. $KMnO_4$

The data show that the rubber membrane, under the experimental conditions stated in the foregoing, has been entirely impermeable to "humic acids." The effect of the HCl treatment of the soil suspension during the b-interval on the liberation of "humic acids" from the soil studied is indicated in the great difference between the amounts used for oxidation of these acids in the two fractions.

The results concerning the cations liberated from the same soil are also given in table 4. No Fe + Al are found in any of the fractions. The electrodialysis of the sample was continued only until about pH 5 was reached in the suspension. It was found in earlier experiments that the liberation of Fe and Al takes place only when the reaction of the suspension has changed to below pH 5 (6).

As seen from the table calcium is found in the largest amounts in the C. II fractions but small amounts are also found in the fractions from C. I. The presence of Ca in C. I can easily be understood, as the liberation of Ca from the suspension still was proceeding when the cathode chambers were drained. In order to obtain complete movement of Ca to the end chamber, the middle compartment should be drained and refilled with distilled water some time before the end chambers are drained.

Magnesium was found in about equal quantities in all fractions, whereas potassium and sodium migrated readily to C. II. The trace of K + Na which was detected in C. I in the b-fraction was presumably there for the same reason as mentioned for Ca.

Silica was found to be present in all the cathode fractions. These results show that the parchment membrane used between C. I and C. II has not prevented the penetration of the silica complexes such as found in the case where rubber membrane was used on the cathode side (experiment with Bedford red clay).

Comparisons of the sums of cations found by analysis and the amounts found by titration gave relatively good agreement. These data also can be found in table 4.

The experiment with the Saw grass muck soil was repeated, the only difference in the experimental conditions being that a cellophane membrane substituted the rubber membrane between A. I and A. II. In this case the dialyzate in A. II becomes brownish in color presumably because of "humic acids," which, in this experiment, moved to the end chamber.

The result obtained in the experiment with the muck soil is of great interest. If it is not possible to provide a membrane more permeable to phosphoric acid

but not permeable to "humic acids," it might be possible to produce pure "humic acids" by continued electrodialysis of the dialyzate from A. I, either in the same experiment (cf. the remarks to experiment 4, table 3), or in the three-compartment cell using rubber membrane on the anode side. Traces of silica apparently can be removed in the same way and the problem of producing the more or less hypothetical "humic acids" in pure form should be solved.

It also should be pointed out that the method of electrodialysis according to the principles developed in the foregoing might be very useful in the study of the different "humic acids" in the soil. By fractional electrodialysis the different acids which are liberated from the soil suspension at various pH values can be studied, and the effect of various treatments in the apparatus on the speed of acid liberation can easily be investigated.

Experiments with electrodialyzed colloidal clay later saturated with certain cations

In a series of experiments the removal and separation of ions from electrodialyzed colloidal Paulding clay later saturated with different cations were studied. The clay suspension, which was prepared and supplied by Dr. R. Bradfield, had a pH value of 3.45, contained 4.3 per cent of clay colloids, and the total base saturation capacity was known to be approximately 80 m.e. per 100 gm. of clay. The amount of suspension used in the experiments corresponded to 2 gm. of clay colloids, which were saturated with 6 parts of either Ca, Na, or K and with 2 parts of either Fe, Al, or Mg. The three first mentioned cations were added in the form of hydroxides, the three latter as solutions of the corresponding chlorides. In one case the Mg was added as oxide.

After being mixed the samples were shaken for 2 hours in a shaking machine and afterwards stored over night. Before pouring into the electrodialysis apparatus the samples were shaken thoroughly by hand, and the pH values determined. The suspension in the electrodialysis apparatus was thoroughly stirred and a constant temperature of 25°C. was maintained during the time of electrodialysis. The experiments were continued until the original pH value of the suspension was approximately reached.

The membrane arrangement in the experiments as well as the results are reported in tables 5, 6, 7, 8, and 9. The experiment reported in table 9 was performed with a four-compartment apparatus, as only one anode chamber was used, while the other experiments were made with the five-compartment cell, the two compartments at each side of the central chamber being used.

Table 5 gives the results with the Ca and Fe saturated clay. Practically all the Ca put into the soil suspension was found in C. II, and no trace was detected in C. I. The Fe liberated, however, was stopped in chamber C. I except possibly a trace in C. II. Only about half of the amount of Fe, however, which was used for saturation, was found in C. I. It should be noted that a considerable amount of Fe precipitate was found on and in the membrane between the middle chamber and C. I. Traces of silica were detected in all

TABLE 5

Experiment with electrodialyzed clay colloids later saturated with calcium and ferric ions and then electrodialyzed for 24 hours

CATHODE CHAMBERS				MIDDLE CHAMBER	ANODE CHAMBERS				pH OF THE SOIL SUSPENSION
C. II	Membrane	C. I	Membrane		Membrane	A. I	Membrane	A. II	
m.e.		*m.e.*				*m.e.*		*m.e.*	
Ca = 1.174 Si (trace)	Parch-ment	Fe (+Al) = 0.176 Si (trace)	Parch-ment	Clay suspension 1.200 m.e. Ca in $Ca(OH)_2$ 0.400 m.e. Fe in $FeCl_3$	Cello-phane	Si (trace) Fe (+Al) (trace) Titratable acids = 0.017	Cello-phane	Cl = 0.293 Si (trace) Fe (+Al) (trace) Titratable acids = 0.300	Initial 5.45 Final 3.45

TABLE 6

Experiment with electrodialyzed clay colloids later saturated with sodium and aluminum and then electrodialyzed for 24 hours

CATHODE CHAMBERS				MIDDLE CHAMBER	ANODE CHAMBERS				pH OF THE SOIL SUSPENSION
C. II	Membrane	C. I	Membrane		Membrane	A. I	Membrane	A. II	
m.e.		*m.e.*				*m.e.*		*m.e.*	
Na = 1.199 Si (trace)	Parch-ment	Al (+Fe) = 0.131 Si (trace)	Parch-ment	Clay suspension 1.200 m.e. Na in NaOH 0.400 m.e. Al in $AlCl_3$	Cello-phane	Si = 0.299 Al (+Fe) (trace) Titratable acids = 0.030	Cello-phane	Cl = 0.232 Si (trace) Al (+Fe) (trace) Titratable acids = 0.303	Initial 6.40 Final 3.60

TABLE 7

Experiment with electrodialyzed clay colloids, later saturated with potassium and magnesium and then electrodialyzed for 48 hours

CATHODE CHAMBERS				MIDDLE CHAMBER	ANODE CHAMBERS				pH OF THE SOIL SUSPENSION
C. II	Membrane	C. I	Membrane		Membrane	A. I	Membrane	A. II	
m.e.		*m.e.*				*m.e.*		*m.e.*	
K = 1.199 Mg (trace) Fe + Al (trace) Si (trace)	Parchment	Mg = 0.061 Fe + Al = 0.131 Si (trace)	Parchment	Clay suspension 1.200 m.e. K in KOH 0.400 m.e. Mg in $MgCl_2$	Cellophane	Si (trace) Fe + Al (trace) Mg (trace) Titratable acids = 0.030	Cellophane	Cl = 0.244 Si (trace) Mg (trace) Titratable acids = 0.252	Initial 5.25 Final 3.50

TABLE 8

Experiment with electrodialyzed clay colloids, later saturated with potassium and magnesium and then electrodialyzed for 140 hours

CATHODE CHAMBERS				MIDDLE CHAMBER	ANODE CHAMBERS				pH OF THE SOIL SUSPENSION
C. II	Membrane	C. I	Membrane		Membrane	A. I	Membrane	A. II	
m.e.		*m.e.*				*m.e.*		*m.e.*	
K = 1.175 Mg (trace) Fe + Al (trace) Si (trace)	Parchment	Mg = 0.069 Fe + Al = 0.131 Si = 0.139	Parchment	Clay suspension 1.200 m.e. K in KOH 0.400 m.e. Mg in MgO	Cellophane	Si (trace) Fe + Al (trace) Mg (trace) Titratable acids = 0.010	Cellophane	Si (trace) Fe + Al (trace) Mg (trace) Titratable acids = 0.035	Initial 5.20 Final 3.65

TABLE 9

Experiment with electrodialyzed clay colloids later saturated with potassium and magnesium and then electrodialyzed for 6 hours

CATHODE CHAMBERS				MIDDLE CHAMBER	MEMBRANE	ANODE CHAMBER	pH IN THE SOIL SUSPENSION
C. II	Membrane	C. I	Membrane				
m.e.		*m.e.*				*m.e.*	
K = 1.199 Mg (trace) Fe + Al (trace) Si (trace)	Cellophane	Mg (trace) Fe + Al (trace) Si (trace)	Parchment	Clay suspension 1.200 m.e. K in KOH 0.400 m.e. Mg in $MgCl_2$	Cellophane	Cl = 0.157 Si (trace) Fe + Al (trace) Mg (trace) Titratable acids = 0.151	Initial 5.22 Final 3.50

fractions and in the anode fractions were also detected significant traces of (Fe + Al). The amounts of titratable acids in the anode fractions are also given in the table. It will be seen that the amount of Cl in the A. II fraction accounts for the main part of the acids found in that chamber. The reason why a smaller amount of Cl is found in the anode water than the amount which was added in the $FeCl_3$ solution is that some of the Cl was liberated as gas at the anode. Practically no acids were found in A. I.

The experiment with the Na and Al saturated clay is reported in table 6. As the data easily can be studied in the table, and show principally results similar to those in the previously described experiment no further comment should be necessary.

The experiments recorded in tables 7 and 8 deal with K and Mg saturated clay. The experimental conditions were very much the same in the two cases, the only difference being that Mg was added as MgO in the latter instead of $MgCl_2$.

It is of interest to note that it required a much longer period of time to reach a low pH value in the case where MgO was used (table 8). In both experiments traces of Fe + Al and of Si were detected in all chambers. The slightly alkaline or neutral reaction in C. I has, in other words, not been sufficient to precipitate all Fe and Al as the respective hydroxides. As may be noted, traces of Mg were detected in C. II. The amounts found in C. I were very small in comparison to the amounts used for saturation. On the other hand, Mg seems to have a rather high replacing effect against Fe and Al, which were found in about the same amounts in these experiments as the amounts found when Fe or Al were used as saturation agents (table 5 and 6). It should here be stated that tests made showed that practically all the Fe + Al found in the Mg experiments consisted of Fe.

Table 9 gives the set-up and results of the experiment with the four-compartment cell. As membrane between C. I and C. II cellophane was used in order to determine whether the slightly lower permeability to cations which this type of membrane shows compared with parchment, would be sufficient to prevent the penetration of Fe, Al, and Mg under the existing experimental conditions. The experiment was continued for only 6 hours, which proved to be sufficient to reach the same pH value as in the experiment reported in table 7.

No trace of K was found in the intermediate chamber, but all had migrated to C. I. The membrane arrangement used, however, was not entirely satisfactory for a complete retention of Fe, Al, and Mg from the end chamber, since traces of these ions were detected in C. II. The amounts of Fe, Al, and Mg, however, which migrate to the second chamber from the central compartment seem to be very small even when the membrane which separates the two chambers is permeable to these ions. Especially is that found to be the fact when we deal with Fe and Al. This is not only the case in the last reported experiment but similar results may be noted when all experiments are taken into consideration.

SUMMARY

This paper deals with the possibility of an electrodialytical separation of ions bearing similar electrical charges.

A new five-compartment electrodialysis apparatus is presented.

Experiments performed indicate that a separation of certain ions can be made by using membranes of different permeability between the chambers which are placed on the same side of the central compartment.

Experimental results indicated that a separation of certain cations from other cations can apparently be made also by chemical means, as a precipitation can be arranged in an intermediate chamber, whereas other cations are allowed to migrate to the end chamber.

A method of producing pure "humic acids" is suggested.

It was found that Fe, Al, and Mg under certain conditions migrated to the anode, presumably in the form of complexes consisting of hydroxides which had absorbed acid anions.

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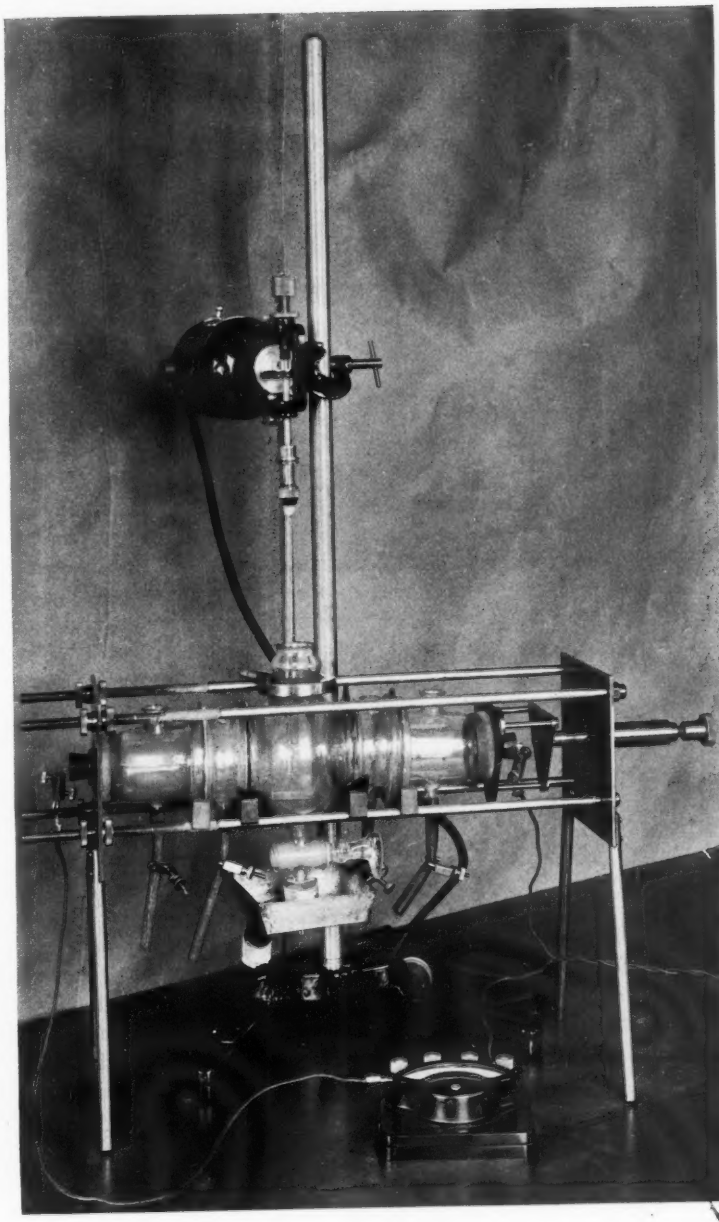
PLATE 1

FIVE-COMPARTMENT ELECTRODIALYSIS APPARATUS

PLATE 1



INTERACTION BETWEEN AMMONIA AND SOILS AS A NEW METHOD OF DETERMINING THE STATE OF SATURATION AND pH VALUES OF SOILS

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In earlier publications (5, 6, 7), the writer has shown that the interaction between soil and ammonia represents the neutralization of acidoid by a base. It was also shown that a soil made completely unsaturated can combine with an amount of ammonia which may be considered equivalent to its base exchange capacity (7); also that the pH value of soils containing excess of $CaCO_3$, after dilute acid treatment and ammonia saturation, is approximately the same as that of the soil in the natural condition. In other words the titration curves of soil acidoids with $Ca(OH)_2$ and NH_4OH are almost identical.

Since pH values of natural soils represent single points on their titration curves, it is reasonable to suppose that the difference between the amount of ammonia taken up by a natural soil and the same soil completely unsaturated, will be a measure of the state of saturation of that soil and consequently will be correlated with its pH value. The object of this investigation was to explore the possibilities of this reaction for characterizing the state of saturation of soil.

EXPERIMENTAL

Ammonia reaction

The amount of ammonia that a natural soil can take up is determined as follows:

Ten to twenty grams of soil are taken in a dish and kept over normal ammonia in a vacuum desiccator for 2 days. It is then transferred to another desiccator and kept over 90 per cent H_2SO_4 for 2 days. The ammonia retained by the soil is then determined by distillation with lime in the usual way.

The maximum saturation capacity of the soil for ammonia is determined as follows:

Ten grams of soil is stirred with about 50 cc. of water. Enough 0.2*N* HCl to break up all the carbonates is added gradually and the mixture set aside with

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frequent shaking till no more bubbles of CO_2 are formed. The soil is then transferred to a filter paper and washed with 0.05*N* HCl till the filtrate shows no reaction for Ca ions; this is followed by leaching with water till the soil is free from Cl ions. It is then washed with a little alcohol and air dried. The final washing with alcohol makes the soil loose and friable as well as easily detachable from the filter paper.

For washing purposes, a Buchner funnel is generally convenient, though a better method is to use the filtering apparatus described by the writer elsewhere (3), with the modification that the cylinder portion need be only 2 or 3 inches long and provided with a lip.

After being air dried the soil is transferred to a flat dish. If a hardened filter paper, like the Watman 50, is used the soil comes off quantitatively, with the help of a spatula and camel's hair brush. The soil is then kept over normal ammonia and subsequently over 90 per cent H_2SO_4 exactly as in the case of natural soil.

The state of saturation of the soil (V) is calculated from the formula

$$(V) = \frac{100\ (T - S)}{T}$$

where S and T are the amounts of ammonia taken up by the natural and acid treated soil respectively. All quantities, of course, are expressed in equivalents

pH value measurements

The greatest difficulty in correlating any physico-chemical measurement on soils with their pH values, lies in the choice of the method for the determination of the latter. The fact that whereas all methods more or less agree among themselves in the case of buffer solutions, they show wide variations in the case of soils is clear evidence that the presence of the soil introduces a variable that is by no means constant or determinable. For the present investigation it appeared necessary to make measurements with all the available methods, in order to see which of them correlates best with the state of saturation of the soil as determined by the ammonia method.

Fifty-seven soils from various parts of India were examined for their reaction by the following methods:

1. Hydrogen electrode 1:5 soil-water ratio, 2 hours' shaking (H).
2. Quinhydrone 1:5 soil-water ratio, 2 hours' shaking (Q).
3. Quinhydrone 1:1 soil-water ratio, 2 hours' shaking (Q′).
4. Quinhydrone 1:1 soil-water ratio, 2 hours' shaking and leaving overnight (Q″).
5. Antimony electrode 1:1 soil-water ratio, 2 hours' shaking and leaving overnight (Sb).
6. Colorimetric method 1:5 soil-water ratio, occasional stirring, and leaving overnight (C). Clear solution obtained by filtering through the percolating cylinder described by the author (3).

The results given in table 1 indicate the magnitude of variations to be expected in the different methods, and as a result of slight alterations in the

technique. The relation between (V) and various sets of pH values is brought out best by working the correlation coefficients between them. These are given below:

(V) and (H) = 0.897
(V) and (Sb) = 0.891
(V) and (Q'') = 0.889
(V) and (C) = 0.938

It will be seen that the correlations are highly significant. The difference is not very great but with the colorimetric method slightly better correlation is obtained than with the other methods. The last column in table 1 gives the pH values calculated from (V) values by the following emperical formulas:

1. pH = 0.0274 (V) + 5.4 for (V) values below 45 per cent
2. pH = 0.0319 (V) + 5.89 for (V) values between 45–81 per cent
3. pH = 0.0319 (V) + 7.11 for (V) values above 81 per cent

The difference between the calculated values (C') and those obtained by the colorimetric method, except for soils having (V) above 81 per cent, are also recorded in table 1. For the latter, the calculated values are compared with the H electrode, and recorded in the same table. The magnitude of these differences except in the case of half a dozen soils, is not greater than what might be expected from the nature of such measurements, and is of the same order as differences between measurements of pH values by any two methods. The calculated values of pH are therefore probably just as reliable as those obtained by any direct method. It might be mentioned that of the soils that show the greatest discrepancy, P.C. 15 and P.C. 32 contain a large amount of humus. Soils with low T values like P.C. 25 are likely to have considerable error in the determination of (V).

In working out the correlations, soils having (V) above 81 per cent were left out, as these obviously fall in a separate group. Nor can it be expected that a linear relationship will hold good for the whole range, for that would mean that the titration curve of a soil is a straight line, which it is not. Besides, the quinhydrone values for highly saturated soils are not reliable; they are merely recorded for comparison.

Apart from the inherent weakness that a specified technique must be followed for the measurement of pH values, it is a well-known fact that such values when found need careful handling to prove of practical utility. There is, for instance, no significant correlation between pH values and lime requirement of soils. The limit of toleration of plants lies within wide ranges of pH values so that an approximate value is all that is necessary to find. The determination of (V) values, on the other hand, will give far more valuable information regarding a soil, at the same time giving an approximate idea of its reaction.

Some very interesting facts emerge from a closer study of the (V) values for various soils. It is found that all soils having (V) values less than 33.3 per

TABLE 1

The relation between the state of saturation (V) and the pH value of soils

SOIL LABORATORY NUMBER P.C.	LOCALITY	CLAY 0.002 MM.	T (PER 100 GM. SOIL)	(V)	pH (H)	pH (Sb)	pH (Q)	pH (Q′)	pH (Q″)	pH (C)	pH (C′)	(C) − (C′)
				per cent								
9	Malabar	26.1	8.1	0	5.76	5.86	5.60	5.47	5.46	6.0	5.4	+0.6
12	Tocklai (Assam)	7.2	4.6	0	5.84	5.50	5.12	4.87	4.93	5.2	5.4	−0.2
14	Madras	21.5	17.2	0	5.37	5.54	5.24	5.16	5.16	5.5	5.4	+0.1
6	Dacca (Bengal)	28.4	11.0	23.6	5.29	5.28	5.16	4.97	4.92	5.1	6.05	−0.95
20	Burma	8.1	4.4	27.3	5.64	6.39	5.58	5.32	5.56	6.4	6.15	+0.25
37	Rangpur (Bengal)	4.1	7.6	30.3	5.75	6.02	5.72	5.50	5.58	6.6	6.23	+0.37
15	Shillong (Assam)	22.4	14.3	31.5	7.71	7.31	7.07	6.74	6.73	7.6	6.26	+1.37
32	Tharavoor (Travencore)	64.6	43.9	31.7	5.05	5.39	5.02	4.73	4.83	5.6	6.27	−0.67
18	Burma	22.6	11.7	33.3	5.79	6.56	5.53	5.32	5.30	6.6	6.31	+0.29
25	United Provinces	4.0	2.1	33.3	7.41	7.52	7.24	7.28	7.14	7.2	6.56	+0.89
22	Burma	15.1	12.7	42.5	6.85	6.56	6.60	6.40	6.46	7.0	7.49	−0.44
19	Mandelay	42.3	24.05	50.1	8.40	7.72	8.28	8.03	7.87	8.0	7.52	+0.51
45	Bihar	11.1	5.8	50.9	7.47	7.87	7.84	7.82	7.64	7.7	7.55	+0.18
49	Madhupur (Bengal)	23.3	17.0	51.8	6.33	6.28	6.49	6.12	6.17	7.0	7.57	−0.55
21	Burma	13.5	12.9	52.7	8.25	7.78	8.01	7.83	7.75	7.95	7.60	+0.38
23	Burma	11.3	11.1	53.6	7.41	7.29	7.29	7.10	7.15	7.5	7.60	−0.10
11	Coimbatore (Madras)	32.8	23.7	54.0	8.77	8.74	8.82	8.64	8.54	8.0	7.62	+0.38
48	Montgomery (Punjab)	19.8	4.7	55.3	8.55	8.48	8.39	8.53	8.29	8.2	7.66	+0.54
46	Baroda (Bombay)	56.4	51.2	56.3	7.63	7.32	7.91	7.42	7.20	7.7	7.69	+0.01
3	Dharwar (Bombay)	62.2	63.1	57.8	7.64	7.14	8.25	7.62	7.29	7.3	7.74	−0.44
44	Bihar	8.4	6.5	59.2	8.54	8.58	8.30	8.43	8.28	7.9	7.78	+0.12
35	Gurgaon (Punjab)	18.3	11.6	59.5	7.98	7.85	8.12	8.06	7.97	7.7	7.79	−0.09
10	Coimbatore	35.6	25.1	60.2	8.71	8.58	8.78	8.54	8.46	8.1	7.81	+0.29
13	Nagpur (C.P.)	58.9	48.0	60.2	8.53	8.05	8.78	8.34	8.23	7.7	7.81	−0.11
34	Gurdaspur (Pb.).	11.3	7.0	60.7	7.63	7.38	7.64	7.49	7.45	7.2	7.83	−0.63
29	Jubbolpur (C.P.).	63.0	49.1	61.1	8.11	7.75	7.72	7.63	7.53	7.9	7.84	+0.06

5	Punjab	12.3	7.32	61.75	8.77	9.12	8.77	8.93	8.88	7.9	7.86	+0.04
42	Bombay	53.4	56.3	62.2	9.00	8.45	8.93	8.47	8.47	8.3	7.88	+0.42
31	Travancore	22.8	17.1	62.6	9.01	7.58	7.83	7.53	7.54	7.7	7.89	−0.19
40	Bombay	13.1	11.0	62.7	7.65	7.48	7.69	7.57	7.51	7.7	7.89	−0.19
43	Bihar	21.6	15.3	62.7	8.41	8.54	8.38	8.31	8.12	8.1	8.20	−0.10
41	Poona (Bombay)	53.4	51.7	63.1	8.74	8.44	8.87	8.60	8.45	8.2	7.91	+0.29
26	Hebbal (Mysore)	22.5	5.7	63.2	8.11	7.90	7.72	7.63	7.53	7.8	7.91	−0.11
50	Ternab (N.W.F.)	17.7	9.6	63.5	8.54	9.00	8.67	8.77	8.71	8.3	7.92	+0.38
38	Broach (Bombay)	52.9	47.3	63.8	8.29	8.21	8.59	8.26	8.30	8.0	7.93	+0.07
8	Coimbatore	25.2	18.7	64.2	8.41	8.42	8.51	8.32	8.31	7.7	7.94	−0.24
17	Kalyanpur (U.P.)	14.1	8.2	64.4	8.20	7.84	8.12	7.99	7.90	8.0	7.95	+0.05
52	Sabour (Bihar)	11.3	6.2	64.5	8.02	8.35	8.01	8.06	7.99	7.6	7.95	−0.35
4	Punjab	15.2	8.2	64.6	8.55	8.66	8.76	8.83	8.77	8.85	7.95	−0.10
16	Lyallpur (Pb.)	8.7	4.9	65.5	8.74	8.38	8.35	8.45	8.33	8.1	7.98	+0.12
1	Pusa (Bihar)	11.3	4.8	66.0	8.41	8.19	8.08	8.13	7.90	8.1	8.00	+0.10
2	Akola (Bombay)	59.3	57.1	66.8	8.21	7.73	8.45	8.15	7.80	8.1	8.02	+0.08
51	Tarnab (N.W.F.)	22.2	7.6	67.1	8.68	8.94	8.62	8.69	8.62	9.0	8.03	+0.97
24	Meerut (U.P.)	9.7	8.8	68.2	8.59	8.69	8.34	8.42	8.30	8.2	8.07	+0.13
27	Mysore	53.2	51.3	69.4	9.03	8.40	9.01	8.74	8.53	8.3	8.11	+0.19
30	Hoshangabad (C.P.)	54.1	57.5	69.9	8.45	8.23	8.74	8.36	8.38	8.1	8.12	−0.02
39	Nadiad (Bombay)	8.4	11.9	72.3	9.11	8.82	8.63	8.71	8.46	8.1	8.20	−0.10
36	Hansi (Punjab)	11.7	6.85	74.5	8.46	8.48	8.33	8.43	8.31	8.1	8.27	−0.17
54	Montgomery (Punjab)	3.2	2.8	78.6	8.71	8.08	8.50	8.54	7.92	8.0	8.40	−0.40
56	Sakrand (Sindh)	13.1	4.9	79.6	8.53	8.72	8.29	8.19	8.10	8.5	8.45	+0.07
55	Sakrand (Sindh)	11.0	7.8	80.8	8.36	8.41	8.12	7.94	7.75	8.1	8.47	−0.37
												(H) − (C′)
47	Montgomery	17.1	6.6	81.8	9.98	9.86	8.76	9.02	8.71	8.7	9.71	+0.27
53	Montgomery	17.6	6.0	85.0	9.87	9.10	9.10	9.04	8.89	....	9.81	+0.06
7	Montgomery	21.8	7.1	90.0	9.58	9.27	8.88	8.86	8.88	9.2	9.97	−0.39
60	United Provinces	7.4	7.3	95.9	10.19	10.35	9.73	9.79	9.89	....	10.16	+0.03
57	United Provinces	4.4	9.0	96.7	10.40	10.20	9.39	9.22	9.02	....	10.19	+0.21
58	United Provinces	13.4	4.7	100.0	10.36	10.78	9.86	9.95	10.13	....	10.29	+0.06

cent respond to lime, the calculated pH value for which is about 7. The neutral point, or pH 7, therefore, has a more fundamental significance than has been hitherto supposed. Most of the good agricultural soils have (V) values between 50 and 70 per cent. Soils having (V) values above 70 per cent invariably contain exchangeable sodium, and above 80 per cent are all barren alkali soils. Soils that were in approximate equilibrium with $CaCO_3$ under field conditions gave values about 62–68 per cent. All soils having (V) values less than 40 per cent are from humid regions where the rainfall is plentiful, and those with above 70 per cent (V) values are from arid regions where the rainfall is scarce.

If 33.3 per cent saturation is taken as the basis for lime requirement, the ammonia method will give values comparable with the pH method of Hardy and Lewis (1) for the lime requirement of soils, which consists in bringing the soil to pH 7. If, on the other hand, the values of lime requirement are to be compared with the methods of Hutchinson, or Kappen, which are equivalent to bringing the soil to pH 6.5 (2), the lime requirement should be calculated to 25 per cent (V) value. Hissink's method for determining (V) values gives the lime requirement at 28 per cent degree of saturation, which is not very different.

The writer has shown elsewhere (5) that the soil acidoid is a tribasic acid containing three equivalents of hydrogen designated as H_1, H_2, H_3. It was also shown that the lime hunger of a soil, from the practical point of view, is satisfied with the neutralization of H_1, which also amounts to 33.3 per cent saturation. It is a remarkable fact that a number of methods of lime requirement of soils should correspond to the same (V) value.

The writer had once expressed the view that soils in equilibrium with $CaCO_3$ should be considered as saturated (6), for this appeared to be the limit beyond which any base added was easily hydrolyzable; although it was recognized that the most comprehensive definition of saturation point would be that according to Hissink. In a later investigation certain soils were found that had to be classed as supersaturated (4). The present method, however, avoids that confusion, and it is now possible to include all types of soils under one scheme of classification according to their state of saturation. Incidently, since most of the alkali soils contain bicarbonates, Hissink's method of finding their state of saturation would not be applicable, because a part of $Ba(OH)_2$ will be precipitated and shown as absorbed.

It is evident that any method which aims at giving 100 per cent saturation for soils in equilibrium with $CaCO_3$ will require the soil to be brought to 50 per cent saturation for finding the lime requirement, and the results could be made comparable to those with the ammonia method by a simple calculation.

SUMMARY

Interaction between ammonia and soils, before and after dilute acid treatment, affords a means of characterizing the state of saturation of soils.

From the values of degree of saturation thus obtained, it is possible to calculate the lime requirement as well as the approximate pH value of a soil.

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